Virologisches Institut
der Vetsuisse-Fakultät Universität Zürich

Direktor: Prof. Dr. sc. nat. ETH Cornel Fraefel

Arbeit unter wissenschaftlicher Betreuung von
Dr. med. vet. Jakub Kubacki

Virome of Swiss bats

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Isabelle Simone Hardmeier

Tierärztin
von Zumikon ZH

genehmigt auf Antrag von

Prof. Dr. sc. nat. Cornel Fraefel, Referent
Prof. Dr. sc. nat. Matthias Schweizer, Korreferent

2021

Contents

Zusammenfassung 4

Abstract 5

Submitted manuscript 6

Acknowledgements .....

Curriculum Vitae .....

Zusammenfassung

Viele der kürzlichen Krankheitsausbrüche in Menschen hatten eine zoonotische Virusätiologie. Fledermäuse sind als Reservoirs für eine Vielzahl von Viren mit Potential für artenübergreifenden Infektionen bekannt. Um das Risiko einer solchen Übertragung durch Fledermäuse in der Schweiz zu beurteilen, haben wir das Virom in Gewebe- und Kotproben von 14 einheimischen und 4 migrierenden Fledermausarten bestimmt. Total wurden Genome von 39 verschiedenen Virusfamilien entdeckt, 16 davon die Vertebraten infizieren. Ganze oder partielle Genome von Coronaviren, Adenoviren, Hepeviren, Rotaviren A und H, und Parvoviren mit potentiell zoonotischem Risiko wurden identifiziert. In einer Bodenkotprobe einer *Vespertilio murinus* Kolonie wurde das partielle Genom eines Middle East respiratory syndrome-related Coronavirus (MERS-CoV) mittels Next generation sequencing nachgewiesen und mittels PCR bestätigt. Die meisten nachgewiesenen Genome gehörten zu Viren welche Insekten infizieren, was die insektenfressende Ernährung der untersuchten Fledermäuse reflektiert. Schlussfolgernd tragen Fledermäuse in der Schweiz natürlicherweise viele verschiedene Viren in sich. Metagenomische Analysen von nicht invasiven Proben wie Bodenkotproben könnten eine effektive Überwachung und frühe Erkennung von viralen Zoonosen unterstützen.

Keywords: Next-generation sequencing, Metagenom-Analyse, Fledermaus-Virom, Middle East respiratory syndrome-related Coronavirus

Abstract

Many recent disease outbreaks in humans have zoonotic virus etiology. Bats have been recognized as reservoirs to a variety of viruses with the potential to cross-species transmission. In order to assess the potential risk of bats in Switzerland for such transmissions, we determined the virome of tissue and fecal samples of 14 native and 4 migrating bat species. In total, genomes belonging to 39 different virus families, 16 of which are known to infect vertebrates, were detected. Full-length or partial genomes of coronaviruses, adenoviruses, hepeviruses, rotaviruses A and H, and parvoviruses with potential zoonotic risk were characterized. Most interestingly, in a ground stool sample of a *Vespertilio murinus* colony the partial genome of a Middle East respiratory syndrome-related coronavirus (MERS-CoV) was detected by Next generation sequencing and confirmed by PCR. Most of the genomes detected belonged to viruses that infect insects, thereby reflecting the insectivorous diet of the examined bats. In conclusion, bats in Switzerland naturally harbour many different viruses. Metagenomic analyses of non-invasive samples like ground stool may support effective surveillance and early detection of viral zoonoses.

Keywords: Next-generation sequencing, metagenomics, virome bats, Middle East respiratory syndrome-related coronavirus

Submitted manuscript

The Virome of Swiss bats

Isabelle Hardmeier[1], Nadja Aeberhard[1], Weihong Qi[2], Katja Schoenbaechler[3], Hubert Kraettli[3], Jean-Michel Hatt[4], Cornel Fraefel[1]*, Jakub Kubacki[1]*

[1] Institute of Virology, University of Zurich, Zurich, Switzerland

[2] Functional Genomics Center Zurich, Zurich, Switzerland

[3] Bat foundation Switzerland, Zurich, Switzerland

[4] Clinic for Zoo Animals, Exotic Pets and Wildlife, University of Zurich, Zurich, Switzerland

* cornel.fraefel@uzh.ch (CF), jakub.kubacki@uzh.ch (JK)

Abstract

Many recent disease outbreaks in humans have zoonotic virus etiology. Bats have been recognized as reservoirs to a variety of viruses with the potential to cross-species transmission. In order to assess the potential risk of bats in Switzerland for such transmissions, we determined the virome of tissue and fecal samples of 14 native and 4 migrating bat species. In total, genomes belonging to 39 different virus families, 16 of which are known to infect vertebrates, were detected. Full-length or partial genomes of coronaviruses, adenoviruses, hepeviruses, rotaviruses A and H, and parvoviruses with potential zoonotic risk were characterized. Most interestingly, in a ground stool sample of a *Vespertilio murinus* colony the partial genome of a Middle East respiratory syndrome-related coronavirus (MERS-CoV) was detected by Next generation sequencing and confirmed by PCR. Most of the genomes detected belonged to viruses that infect insects, thereby reflecting the insectivorous diet of the examined bats. In conclusion, bats in Switzerland naturally harbour many different viruses. Metagenomic analyses of non-invasive samples like ground stool may support effective surveillance and early detection of viral zoonoses.

Introduction

Bats belong to the order Chiroptera, a group of mammals with 21 families and more than 1'300 species of which approximately 1'236 are classified by the IUCN [1-4]. Bats are one of the most diverse and abundant groups of animals, living all over the world except the Arctic and Antarctic [5, 6]. Nevertheless, nearly a third of all bat species are endangered [1, 7-9]. Bat species have adapted to various food sources such as arthropods, fruit, nectar, pollen, small mammals, fish, frogs, and blood, and they found a niche to navigate and hunt in darkness with the ability of echolocation and their ability to fly [10]. Bats play a key role in the ecosystems by pollinating flowers and dispersing seeds [10-12]. Five different families of insectivorous microbats are endemic in Europe, including Vespertilionidae, Molossidae, Rhinolophidae,

Pteropodidae and Emballonuridae. Of the approx. 53 bat species identified in Europe, 30 are also endemic in Switzerland [5-7, 13, 14].

Bats have unique biological, physiological, and immunological characteristics that seem to make them ideal candidates to host and spread viruses. First of all, they are the only mammals capable of powered flight, some species migrate several hundred miles for winter hibernation enabling viral spread over long distances [15-17]. In contrast stands the "reservoir richness hypothesis" which assumes that the number of different bat-associated zoonotic viruses is more likely due to the larger number of detected bat species ("species richness") than to the special characteristics of bats mentioned above [18, 19]. In Switzerland, some species are known to fly long distances including *Nyctalus noctula*, *N. leisleri* and *N. lasiopterus*, *Vespertilio murinus* and the *Pipistrellus nathusii* [7]. Secondly, the longevity, i.e. up to 41 years (*Myotis brandtii*), facilitates long virus persistence [20-23]. Thirdly, bats roost in colonies with a size of up to a million animals per colony facilitating virus transmission between individuals [16]. Moreover, as bats are phylogenetically the oldest mammals on earth, their immune system was able to adapt and cope with viral infections over a long time of co-evolution. Bats seem to control viral replication more effectively than other mammals [24, 25]. It has been shown that due to the development of flight, the immune system adapted to the high metabolic rates i.e., increased metabolism and high body temperature during flight, through genetic changes. Flight would therefore lead to a daily activation of the immune system and could explain why bats can host viruses with no signs of illness [16, 24, 26, 27]. In addition, the large body temperature fluctuations between flight and resting, daily torpor, and hibernation may result in bat derived viruses that are less temperature sensitive (tolerating febrile and cold temperatures) and highly pathogenic for humans and other mammals [26]. During hibernation, with temperatures between 0 and 8 °C, immune responses of bats may be suppressed which allows viruses to persist [16, 28]. At 24 °C, bats have significantly higher antibody levels than during hibernation [16, 27, 29-31]. The neutralizing antibodies of bats

may have a shorter lifespan than in other mammals, a hypothesis that is supported by the fact that bats are re-infected with tickborne encephalitis viruses although neutralizing antibodies specific for this virus had previously been present [16, 32]. Furthermore, in *Eptesicus fuscus*, overexpression of inflammatory genes can be controlled by the innate immune system in response to viral nucleic acid [33]. Indeed, many different viruses have been detected in bats using a variety of assays, including serological tests, polymerase chain reaction (PCR) and virus isolation [16, 32, 34-43].

Bats are not only ideal hosts and reservoirs for many different viruses, but in addition several factors also support the efficient transmission of viruses. These include intrinsic factors such as body condition, sex, or social and reproductive status as well as extrinsic factors influencing the habitat of the bats such as drought, cave destruction, and bush fires [20]. Stress (e.g., starving, mating, breeding, fighting, high population density) amplifies virus transmission from bat to bat even further as it downregulates the immune system and makes the animals more susceptible to viral infections, which has a direct impact on the epidemiology of viral diseases [16, 20, 44-46]. Habitat loss and lack of food often forces bats to move closer to domestic areas, thereby increasing the chance of virus transmission to other species including humans and farm animals.

Bats are the natural hosts for many different emerging zoonotic viruses such as Ebola virus, Marburg hemorrhagic fever viruses, rabies virus, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle-East respiratory syndrome coronavirus (MERS-CoV), Nipah (NiV) and Hendra virus (HeV) [14, 20, 26, 34, 40, 47-50]. Up to date, 20 out of 29 viral families of bat-associated viruses were detected in Europe, and the different viruses were compiled in a database called DBatVir [51, 52]. Viruses from at least 12 viral families found in Europe have zoonotic potential, but direct bat-to-human transmission has been reported in Europe only for lyssaviruses and so far with a low prevalence [14, 34]. For transmission of bat-borne viruses to humans often an intermediate host is needed [49]. For example, horses

with sudden acute respiratory symptoms played an intermediate species role in the transmission of HeV from flying fox bats to humans during a HeV outbreak in 1994 in Australia [53]. The Nipah virus outbreak in Malaysia from 1998-1999 is an example for anthropogenic factors leading to virus emergence. In particular, the pig industry rapidly expanded into the habitat of the natural NiV host, flying foxes, which resulted in the transmission of the virus to pigs. Additionally, pigs served as amplifying hosts and transmitted the virus to humans, where it caused encephalitis and lead to 105 deaths [30, 53]. To stop the further spread of disease, more than a million pigs have been culled.
In the coronavirus outbreaks SARS-CoV and MERS-CoV, the viruses originated from bats but were transmitted to humans through an intermediate host i.e. civets and dromedary, respectively [48, 50, 54, 55]. The pandemic caused by SARS-CoV-2 presumably originated from a bat coronavirus detected in a bat from the province Yunann (SARS-CoV; RaTG13) with a 96% nucleotide similarity to the SARS-CoV-2 [56, 57]. Coronaviruses circulate widely in wildlife and the bat-to-human transmission and the role of intermediate host in transmission is up to date unclear [58]. However, some reports suggest that the Malayan pangolins (*Manis javanica*) may serve as an intermediate host of SARS-CoV-2 [56, 57, 59, 60].
Furthermore, countless viruses from insects, plants, fungi, and bacteria were detected in bats and mainly reflect the dietary habits of the bats [61-63].

While several studies in China, Japan, Myanmar, the USA and France revealed that bats host many different viruses [5, 62, 64-67] no such data is available from Switzerland. Therefore, in a collaboration with the foundation for bat conservation Switzerland and bat care takers, more than 7’000 bats were sampled for this metagenomic study.

Material and Methods

Animals and sample collection

Samples were collected between 2015 and 2020 from bats presented at care centers in the cantons Zurich, Basel, Berne, Grisons, Jura, Neuchatel, and Lucerne, and from selected bat colonies in the cantons Aargau, Jura, Lucerne, Obwalden, Schaffhausen, Schwyz, Solothurn, St. Gallen, and Zurich (Table 1 and Fig 1). On living bats, only non-invasive sampling procedures (feces collection from the floor) were performed. Specifically, 1.5 g to 2.5 g of ground stool from colonies was collected in 15-ml tubes, and 30 mg to 90 mg of fresh feces from individual animals was collected in 2-ml tubes. Organs were collected from bats which were found dead or brought to the bat care centers and due to injuries had to be euthanized by veterinarians at the bat care centers (anesthetized with 20mg/kg Alfaxalone (Alfaxan) SC in the neck area and then after 10min, euthanized with Pentobarbital (Esconarkon) IP (0.1 – 0.2ml / animal). From necropsy, brain, heart, lung, intestine, and spleen combined with liver were collected and stored separately in 2-ml tubes. All samples were stored at -20 °C. Ethical approval was not required while only non-invasive sampling procedures on living bat were performed, and corpses were from natural deaths or from bats that had to be euthanized due to injuries. The euthanasia, thus, was not part of this study.

Fig 1. Locations in Switzerland where samples were collected, and number of animals sampled. Supported by Bing Copyright GeoNames, HERE, MSFT, Microsoft, Wikipedia.

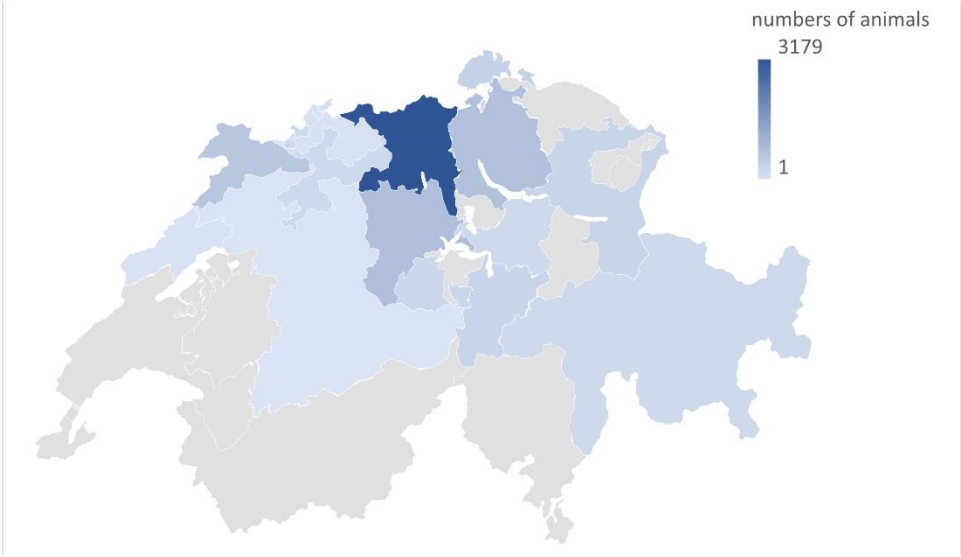


Table 1. Place of collection, bat species, and numbers of the bats and samples investigated in this study.

		Numbers of animals	
Canton	**Bat species**	**Feces**	**Organs**
Aargau	*Myotis daubentonii*	200[a]	
	Myotis myotis	2'454[a]	11
	Myotis mystacinus	50[a]	2
	Myotis nattereri	50[a]	
	Nyctalus noctula	200[a]	
	Pipistrellus nathusii	4	
	Pipistrellus kuhlii	1	
	Pipistrellus pipistrellus		1
	Pipistrellus sp.	1	
	Rhinolophus ferrumequinum	5[a]	
	Vespertilio murinus	200[a]	
Basel	*Pipistrellus kuhlii*		7
	Pipistrellus nathusii		10
	Pipistrellus pipistrellus	1	16
Berne	*Plecotus auritus*		1
Grisons	*Eptesicus nilssonii*		3
	Myotis nattereri		1
	Nyctalus leisleri		2
	Pipistrellus nathusii		1
	Pipistrellus pipistrellus		2
	Plecotus auritus		2
	Plectous macrobullaris		1
	Rhinolophus ferrumequinum	186[a]	
	Rhinolophus hipposideros	12[a]	
Jura	*Myotis myotis*	600[a]	
	Pipistrellus pipistrellus		1
Lucerne	*Myotis daubentonii*	1	
	Myotis myotis	778[a]	1
		2	
	Pipistrellus nathusii		1
	Pipistrellus pygmaeus	1	1
Neuchâtel	*Myotis myotis*	2	
	Pipistrellus nathusii	2	
	Pipistrellus pipistrellus	2	1
Obwalden	*Myotis myotis*	250[a]	
Schaffhausen	*Plecotus austriacus*	1	
	Myotis myotis	370[a]	
Schwyz	*Myotis myotis*	212[a]	
Solothurn	*Myotis myotis*	208[a]	
St. Gallen	*Myotis myotis*	310[a]	
	Plecotus auritus		1
	Pipistrellus kuhlii		1
Uri	*Myotis myotis*	300[a]	
Zurich	*Myotis daubentonii*	4	3
	Myotis myotis	568[a]	
		1	
	Myotis mystacinus	1	1

	Nyctalus noctula	72	
	Pipistrellus kuhlii	8	11
	Pipistrellus nathusii	7	5
	Pipistrellus pipistrellus	13	14
	Pipistrellus sp	7	4
	Plecotus auritus	6	
	Vespertilio murinus	1	2
Liechtenstein	*Myotis myotis*	93[a]	
Total		7'184	107

[a] Pooled fecal sample of colony

Enrichment of viral particles and nucleic acid isolation

Homogenization and filtration

To homogenize fecal samples from individual animals or small groups (up to ten animals), 360 µl of phosphate buffered saline (PBS) was added to 30 mg of feces. To homogenize organs, 300-500 µl of PBS was added to 30 mg of tissue from up to 10 individual animals grouped according to species, geographical location, and organ type. Complete homogenization of tissue samples was achieved by adding a stainless-steel bead of 5 mm diameter (Qiagen, Hombrechtikon, Switzerland) into each tube. Then, samples were homogenized in the TissueLyser II (Qiagen, Hombrechtikon, Switzerland) at 20 Hz for 2 min, followed by 5 min of centrifugation at 16'060 x *g* (Biofuge Fresco, Heraeus Instruments, Hanau, Germany). The supernatants were collected with a 5-ml syringe (Injekt F, B. Braun, Sempach, Switzerland) and a 22 G needle (0.7 x 32 mm, AGANI NEEDLE, Terumo, Eschborn, Germany) and filtered through a 0.45 µm syringe filter (Puradisc 13 mm, Whatman GE Healthcare, Chicago, Illinois, USA) to remove bacterial and host cells. If clogging occurred, filtration was repeated. After the filtration step, pools of tissues from animals of the same species and geographical location were combined in 1.5-ml Eppendorf tubes. Fecal pellets from colonies were placed in a petri dish and cut into pieces with a scalpel blade (carbon steel sterile surgical blade#18, B. Braun, Sempach, Switzerland), and the sample material was divided evenly into two new 15-ml tubes. One aliquot was stored at -20 °C as backup and the other used for homogenization. For this, 12 ml of PBS was added, the sample

mixed with a vortex, and then centrifuged for 10 min at 21'890 x *g* (Hereus Multifuge 3 S-R, Thermo Fisher, Waltham, Massachusetts, USA). The supernatant was transferred into a 2-ml tube and centrifuged a second time for 8 min at 16'060 x *g*. Then, the supernatant was taken out with a 5-ml syringe (Injekt F, B. Braun, Sempach, Switzerland) and 22 G needle (0.7 x 32 mm, AGANI NEEDLE, Terumo, Eschborn, Germany) and filtered first through a 0.8 µm syringe filter (13 mm Supormembrane, Pall Corporation, New York, USA) and then through a 0.45 µm syringe filter (Puradisc 13 mm, Whatman, GE Healthcare, Chicago, Illinois, USA) into 1.5-ml Eppendorf tubes.

Nuclease treatment

To remove nucleic acids not protected by a virus coat, 134 µl of each filtered homogenate from the step above was treated with 1 µl of micrococcal nuclease (2 x 10^6 gel U/ml; New England Biolabs, Ipswich, Massachusetts, USA), 14 µl of 10 X micrococcal nuclease buffer and 1 µl of Ribonuclease A (30mg/ml; Sigma-Aldrich, St. Louis, Missouri, USA). Then, the samples were incubated for 15 min at 45 °C and for 1 h at 37 °C.

Nucleic acid isolation

Viral RNA and DNA was prepared by using the QIAmp Viral RNA Mini kit (Qiagen, Hombrechtikon, Switzerland) as described by the manufacturer with modifications: the RNA carrier was omitted and, as a first step, 594 µl of AVL buffer was mixed with 6 µl of 1% β-mercaptoethanol (Bio-rad, Hercules, California, USA). The nucleic acid was eluted with 20 µl of RNase free water and 20 µl of Tris-EDTA buffer (TE) [68].

Reverse transcription and second strand synthesis

For cDNA synthesis, 2.5 µM of a random primer with a known 20-nt tag sequence (SISPA-N, 5'-GCT GGA GCT CTG CAG TCA TCN NNN NN-3') was added to the nucleic acid samples prepared in the step above, incubated at 97 °C for 3 min, and cooled on ice. Then 20

µl of cDNA-mix (RevertAid First Strand H minus cDNA Synthesis kit; Thermo Fisher, Waltham, Massachusetts, USA), consisting of 10 µl of 5 X reaction buffer, 5 µl of 10 mM dNTP mix, 2.5 µl of 20U/ µl Ribolock RNase Inhibitor, and 2.5 µl of 200U/ µl RevertAid H minus RT, was added to each sample, and the suspension was incubated for 10 min at 25 °C, 90 min at 45 °C, and 5 min at 70 °C. Finally, 1 µl of RNase H (New England Biolabs, Ipswich, Massachusetts, USA) was added to degrade residual RNA, and the sample was incubated for 20 min at 37 °C. For second strand synthesis, 45.5 µl of cDNA, 0.6 µl of 10 X Klenow buffer (Thermo Fisher, Waltham, Massachusetts, USA), 0.4 µl of 100 µM SISPA-N primer, and 1 µl of 10 mM dNTP were mixed, denatured for 1 min at 95 °C and cooled on ice. Then, 2.5 µl of Klenow Fragment 3'→5' exo- (Thermo Fisher, Waltham, Massachusetts, USA) was added, and the mixture was incubated for 15 min at 25 °C and for 1 h at 37 °C and subsequently denatured for 1 min at 95 °C. Following cooling on ice, another 1.25 µl of Klenow Fragment 3'→5' exo- was added and the reaction was allowed to proceed for 15 min at 25 °C and 1 h at 37 °C. Samples were purified using the PureLink® PCR Micro kit (Invitrogen, Thermo Fisher, Waltham, Massachusetts, USA) according the manufacturer's protocol. Finally, dsDNA was eluted with 12 µl of buffer E1 (Invitrogen, Thermo Fisher, Waltham, Massachusetts, USA).

Random Amplification

Before library preparation, dsDNA was amplified using a sequence-independent single primer (SISPA primer, 5'-GTT GGA GCT CTG CAG TCA TC-3') and HotStarTaq DNA polymerase (5U/ µl; Qiagen, Hombrechtikon, Switzerland) as described previously [69]. Then, the samples were purified using the QIAquick PCR Purification kit (Qiagen, Hombrechtikon, Switzerland) according to manufacturer's protocol and eluted with 30 µl of elution buffer (EB). The end concentrations of dsDNA in the samples were measured using a Qubit™ 2.0 Fluorometer (Invitrogen, Carlsbad, California, USA).

Library preparation

For fragmentation of the dsDNA and ligation of specific adaptors to the DNA fragments, the samples were diluted to a final concentration of 3 ng of DNA in 50 µl of EB buffer (or 1 ng per 50 µl for samples with low initial concentrations). The diluted samples were transferred into microTUBES (Covaris, Massachusetts, USA) and sonicated using an S220 Ultrasonicator System (Covaris, Massachusetts, USA) to obtain 500 bp fragments, for paired end NGS runs, or 300 bp fragments, for single end NGS runs, according to the manufacturer's manual. For library preparation, the NEBNext Ultra II DNA Library Prep kit for Illumina (New England Biolabs, Ipswich, Massachusetts, USA) was employed according to the manufacturer's manual. AMPure XP Beads (Beckman Coulter, Brea, California, USA) were used to clean up the adaptor-ligated DNA, and the NEBNext Multiplex Oligos (96 Unique Dual Index Primer Pairs; New England Biolabs, Ipswich, Massachusetts, USA) were used for the barcoding of the samples.

Quality control and sequencing

The Agilent 2200 TapeStation (Agilent Technologies, Santa Clara, California, USA) was used with a D1000 HS ScreenTape (Agilent Technologies, Santa Clara, California, USA) to measure the size distribution and molarity of each sample. Sample denaturation, dilution, and sequencing were performed at the Functional Genomics Center Zurich (FGCZ) on either the NextSeq 500 system using the high-output flow cell with a paired-end NGS run and 2 x 150 nucleotide read length or the Illumina NovaSeq 6000 system using a single end NGS run with 100 nucleotide read length according to the protocols provided by the manufacturer (Illumina, San Diego, California, USA).

Electron microscopy

For electron microscopy, selected fecal and tissue samples were solubilized in 15-ml tubes with 5 to 6 ml of PBS. Then, the same volume of 1-butanol was added prior to vortexing for 3

min and centrifugation for 30 min at 500 - 700 x *g* (Heraeus™ Multifuge™ X3, Thermo Fisher, Waltham, Massachusetts, USA). After incubation at 4 °C for 5 h, the aqueous phase was transferred into a new 15-ml tube and topped up to 5 ml with PBS if the volume was smaller. Then, 5 ml (minimal ratio 1:1) of 1-butanol was added, and the mixture was vortexed for 3 min, centrifugated for 30 min at 500 - 700 x *g* (Heraeus™ Multifuge™ X3, Thermo Fisher, Waltham, Massachusetts, USA), and incubated for 5 h at 4 °C. Again, the aqueous phase was transferred into a new 15-ml tube for mid-speed centrifugation, topped up with PBS to a final volume of 10 ml, and centrifugated for 20 min at 10'000 x *g* (Sorvall RC 5C PLUS using the HB-4 Rotor, Marshall Scientific, Hampton, New York, USA). The supernatant was transferred into an ultra-clear tube (14x95 mm, Beckmann Coulter, Brea, California, USA) and centrifugated for 2 h at 20'000 rpm and 4 °C (60'000 x *g* Sorvall WX 100 ultra-series centrifuge using the SW40 Rotor, Thermo Fisher, Waltham, Massachusetts, USA). Then, the supernatant was discarded, and the pellet containing the viral particles was resuspended in 20 µl of PBS. For negative staining, a parafilm strip (Bemis Company, Inc., Neenah, Wisconsin, USA) was placed onto a smooth surface. Then, 10 µl of the resuspended virus particles, a drop of double distilled water (ddH_2O filtered with 0.22 µm pore size), and a drop of 2% phosphotungstic acid (PTA; H3(P(W3O10)4)xH_2O), pH 7.0 were pipetted side by side onto the parafilm. A grid (carbon coated parlodion film mounted on a 300 mesh/inch copper grid), which was glow discharged to make it hydrophilic, was placed with the carbon coated side onto the top of the sample drop. After 10 min, the grid was placed onto the top of the ddH_2O drop for a few seconds, and finally, onto the PTA for 1 min. Then, PTA was gently removed using a filter paper, and the grid was placed into a transmission electron microscope (CM12, Philips, Eindhoven, Netherlands) equipped with a CCD camera (Ultrascan 1000, Gatan, Pleasanton, California, USA) for analysis at 100 kV.

PCR

Selected viral genomes detected by NGS were confirmed as follows: RNA was prepared by adding 270 µl of PBS to 30 mg of fecal or tissue sample, followed by vortexing and centrifugation for 3 min at 16'060 x *g* (Biofuge Fresco, Heraeus Instruments, Hanau, Germany). Then, 150 µl of the supernatant was used for RNA extraction using the Qiagen RNA Mini kit (Qiagen, Hombrechtikon, Switzerland) according to the manufacturer's manual. DNA from tissue and fecal samples was prepared using the Qiagen DNA Mini kit or the Qiagen DNA Stool kit, respectively, according to the manufacturer's manual (Qiagen, Hombrechtikon, Switzerland).

Pancorona OneStep RT-PCR

The Pancorona OneStep RT-PCR was performed as described by Vijgen et al [70]. The PCR product was analyzed by agarose gel electrophoresis. The expected band of approx. 251 base pairs (bp) was cut out with a scalpel blade, and the DNA was extracted using the Gel extraction kit (Qiagen, Hombrechtikon, Switzerland) according to the manufacturer's manual and sequenced at Microsynth (Balgach, Switzerland).

Panadeno PCR

The Panadeno nested PCR was performed as described by Wellehan et al [71] using 5 µl DNA, 0.4 µl of HotStarTaq Polymerase (5U/ µl, Qiagen, Hombrechtikon, Switzerland), 0.5 µl dNTP mix (10 mM), 2.5 µl of PCR-Buffer (10X), 0.25 µl each of Panadeno outer forward and reverse primer (100 µM) and 16.1 µl nuclease free water. For the second amplification, 2 µl of the reaction mixture from the first amplification were mixed with 0.4 µl of HotStarTaq Polymerase (5U/ µl, Qiagen, Hombrechtikon, Switzerland), 0.5 µl dNTP mix (10 mM), 2.5 µl of PCR-Buffer (10X), 0.25 µl each of Panadeno inner forward and reverse primer (100 µM) and 19.1 µl nuclease free water. The PCR product was then analyzed by agarose gel electrophoresis. The expected band of approx. 318-324 bp was cut out with a scalpel blade,

and the DNA was extracted using the Gel extraction kit (Qiagen, Hombrechtikon, Switzerland) and sequenced at Microsynth (Balgach, Switzerland).

Rotavirus H NSP5 RT-PCR

To amplify the NSP5 segment of Rotavirus H (RVH), specific primers were designed using Primer3Plus [72] with manual inspection in Clonemanager 9 (Sci-Ed Software, Cary, North Carolina, USA). The following primers were used: forward 5'-GGAACTAAAAACTTCAATCGTTGCTG-3' and reverse 5'-GTTTTTATTGATGACCTCAGGGGC-3'. Before amplification, an initial denaturation step with 10 µl of extracted RNA for 5 min at 97 °C was performed. To the denatured RNA 40 µl of the PCR mix containing 10 µl of One Step RT-PCR Buffer (5X; Qiagen, Hombrechtikon Switzerland), 1.6 µl forward Primer (100 µM) and 1.6 µl reverse Primer (100 µM), 2 µl dNTP Mix (10 mM each), 2.0 µl One Step Enzyme Mix (Qiagen Hombrechtikon, Switzerland), 22.6 µl nuclease free water, and 0.2 µl RNasin (Promega, Madison, Wisconsin, USA) was added. The PCR conditions were as follows: 30 min at 50 °C, 15 min at 95 °C, 40 cycles of 50 sec at 94 °C, 50 sec at 55 °C and 60 sec at 72 °C, a final extension step for 10 min at 72 °C. The PCR product was analyzed by agarose gel electrophoresis. The expected band of approx. 666 bp was cut out with a scalpel blade, and the DNA was extracted using the Gel extraction kit (Qiagen, Hombrechtikon, Switzerland) according to the manufacturer's manual and sequenced at Microsynth (Balgach, Switzerland).

Data analysis

Sequencing data was used in reference guided analysis and de novo assembly pipelines, as described previously [68]. First, PCR primers, sequencing adaptors, and low-quality ends in raw reads were trimmed of using Trimmomatic (version 0.36) and cutadapt (version 2.9). Quality controlled reads were aligned using Bowtie2 (version 2.4.1) to the human genome to remove contamination introduced during sample preparation. Un-mapped reads were aligned

to an inhouse database containing 37’400 full length viral genomes using the same aligner, and mapped reads and mapped bases per viral genome were calculated using bedtools (version 2.29.2). Second, the same inhouse database was used to aligned raw reads in the metagenomic pipeline of the SeqMan NGen v17 (DNAStar, Lasergene, Madison, Wisconsin, USA) to visualize and confirm assembled reads. Finally, quality-controlled raw reads were assembled using megahit (version 1.1.3) with multiple k-mers [73]. To annotate contigs taxonomically, assembled contigs were compared against the NCBI nt database [74] using BLASTN (version 2.6.0+) [75] and taxonomically annotated. The resulting viral contigs were further investigated and aligned using MEGA X and Clone manager ver. 9 (Sci-Ed). Phylogenetic trees were constructed in Mega X using the Maximum Likelihood method with 1’000 bootstrap value and a cut-off of 70% [76].

Accession numbers

The nucleotide sequences of selected viruses identified in this study have been registered at the GenBank under the following accession numbers: MT815927-MT815982 and MT818221.

Results

Fresh fecal samples of bats living in Switzerland (single animals, ground stool of colonies) were collected between 2018 and 2020, and tissue samples were taken from dead or diseased bats, who had to be euthanized between 2015 and 2020 (Fig 1). The samples were grouped according to sample type, bat species and collection location into 174 sample pools (69 feces and 105 organ pools) and then sequenced by NGS (Table 1). The data will be presented in the following order (i) a general overview of the virome of Swiss bats, (ii) the virome composition of different bat species, (iii) differences in the virome composition according to the sample types, (iv) an overview of viral genome abundance, and (v) a focus on selected virus families of vertebrates including *Coronaviridae, Adenoviridae*, *Reoviridae, Parvoviridae, Circoviridae,* and *Hepeviridae*.

Overview of the virome of Swiss bats

The raw sequence data consisting of 9.35×10^8 reads (0.5×10^6 to 3.7×10^7 from each pool) were analyzed using two different pipelines: (i) de novo assembly and (ii) reference-based assembly. The de novo assembly generated a total of 7'477 contigs matching to viral genomes. Using an inhouse data base, 1.68×10^7 of the 9.35×10^8 sequenced reads were assembled to virus genomes. Of these, 1.69×10^6 (10%) reads matched to vertebrate viruses and 1.52×10^7 (90%) to non-vertebrate viruses (Fig 2A and 2B). The generated sequencing reads were assembled to 39 virus families, i.e., 16 (41%) vertebrate and 23 (59%) non-vertebrate virus families including 11 (28.2%) families of insect-, 11 (28.2%) families of plant/fungal- and 1 (2.6%) family of environmental viruses (Fig 2B).

Vertebrate viruses. Reads assembled to the genomes of viruses of vertebrates included mainly the families of the *Circoviridae* (84.2%), *Genomoviridae* (3.6%), *Coronaviridae* (3.1%) and *Adenoviridae* (2.6%) (Fig 2A and 2B). Electron microscopy of a ground stool sample of a *Myotis myotis* colony with 1.3×10^6 reads assembled to a Circovirus sp., indeed

revealed particles with a size (approx. 20nm) and shape (icosahedral/round) resembling that of circoviruses (Fig 3A) and an estimated particle concentration of > 10^{12} particles per ml (Fig 3A).

Fig 2. Overview of the virome of Swiss bats. A. Heatmap of reads from 16 virus families of vertebrates and 23 virus families of non-vertebrate viruses and their distribution among all 18 bat species investigated. **B.** Read abundance of the different virus families.

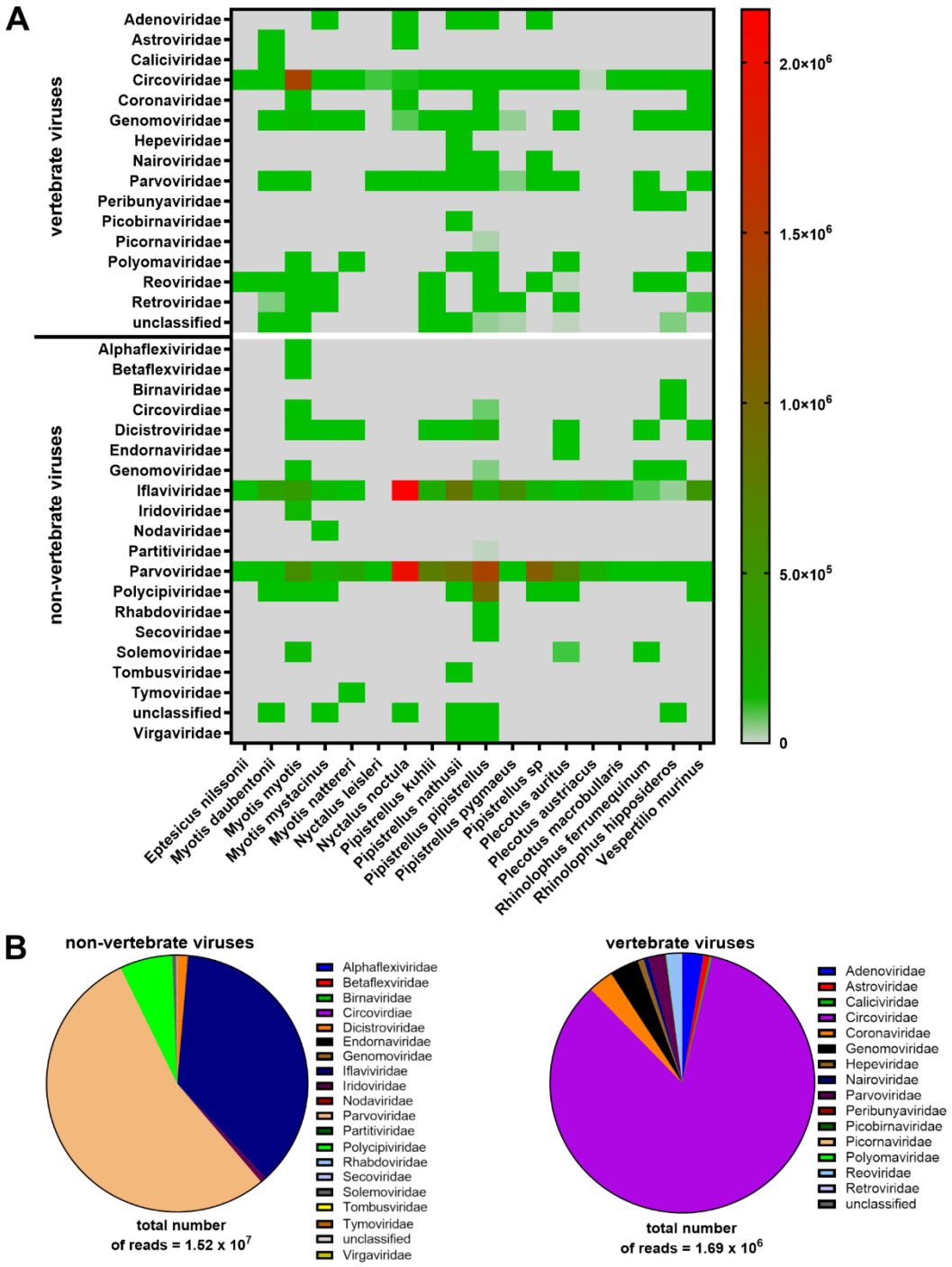
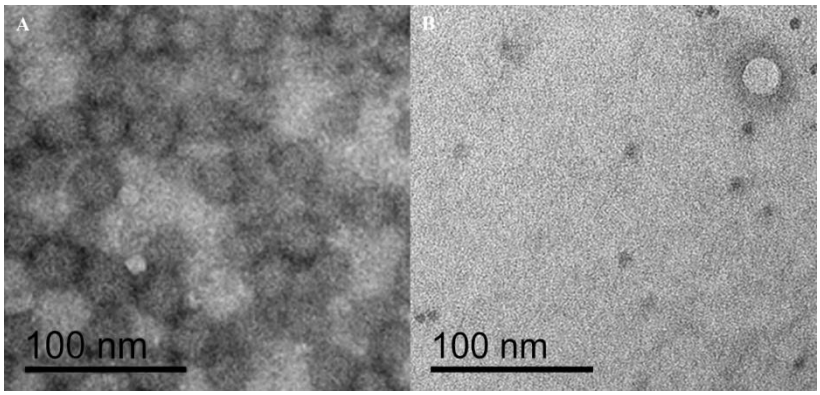


Fig 3. Electron microscopy of selected samples. A. Negative stain electron micrograph of a ground stool pool homogenate form a *Myotis myotis* colony. **B.** Negative stain electron micrograph of a pooled liver and spleen homogenate from *Pipistrellus nathusii.*



Insect viruses. Reads assembled to virus families infecting insects included mainly *Parvoviridae* (54.3%), *Iflaviridae* (37%) and *Polycipiviridae* (6.6%) (Fig 2A and 2B). More than 6 x 10^6 reads were assembled to the subfamily *Densovirinae*, i.e., parus major densovirus, detected in 141 samples. An assembled full-length genome of 5’164 nt from 6 x 10^5 sequencing reads of an intestine pool of *Pipistrellus sp.* had a 97% nucleotide (nt) similarity to a previously described parus major densovirus (GenBank acc. NC_031450). Interestingly, reads assembled to parus major densovirus have been detected also in several tissue pools including lung, liver/spleen, and intestine. Electron microscopy of a pooled liver/spleen homogenate from *Pipistrellus nathusii* tissue revealed virus like particles with a size (approx. 22nm) and shape (icosahedral) resembling that of densoviruses (Fig 3B). Additionally, several bee viruses were detected in ground stool samples of *Myotis myotis* colonies.

Plant/fungal viruses. Reads assembled to genomes of viruses infecting plants or fungi included mainly the families of the *Solemoviridae* (88.1%), *Tymoviridae* (9%) and *Alphaflexiviridae* (1.8%) (Fig 2A and 2B).

The virome composition of the different bat species

The genome of two different virus families were detected in all 18 bat species, the *Circoviridae* and *Densovirinae*. The genomes of *Iflaviridae* were identified in 17, of

Genomoviridae and vertebrate associated *Parvoviridae* in 12, and of *Reoviridae* in 10 bat species. *Pipistrellus pipistrellus* was the bat species with the largest variety of different virus families detected (11 virus families of vertebrates/11 virus families of non-vertebrates; 11/11), followed by *Myotis myotis* (8/10), *Pipistrellus nathusii* (9/7), *Myotis daubentonii* (8/4) and *Plecotus auritus* (7/5) (Fig 2A). Among the 18 species of bats, *Myotis myotis* represented the largest sample group with 87.4% of all sampled animals and was the species with the highest number of reads assembled to vertebrate viruses (87%), as well as the species with the largest number, 33, of different viruses detected (Fig 2A and S1 Fig). The second largest number of different viruses, 24, was detected in *Pipistrellus pipistrellus* (of which 12 were viruses of vertebrates and 12 of non-vertebrates), followed by *Pipistrellus nathusii* and *Myotis mystacinus* with 17 different virus families each.

Five bat species in Switzerland are known to migrate i.e., *Nyctalus leisleri*, *Nyctalus lasiopteros*, *Nyctalus noctula*, *Pipistrellus nathusii* and *Vespertilio murinus*. Samples from all migrating species except *Nyctalus lasiopteros* were collected. Between migrating and non-migrating bats only minor differences in the virus genome diversity and the numbers of reads were observed (S1 Fig). The average numbers of different viruses detected in migrating bats was lower than in non-migrating bats. On the other hand, the average number of different viruses of vertebrates was higher in migrating than in non-migrating bats (Fig 2A and 2B and S1 Fig).

Differences in the virome composition according to the sample types

In general, more reads were detected in fecal and intestine samples than in tissue samples (brain, liver together with the spleen, and lung samples). Highest read numbers assembled to vertebrate virus families were detected in fecal samples from colonies, whereas assembled reads of non-vertebrate viruses were highest in fecal pools of single animals. Especially *Retroviridae* genomes were detected mainly in brain samples (Table 2).

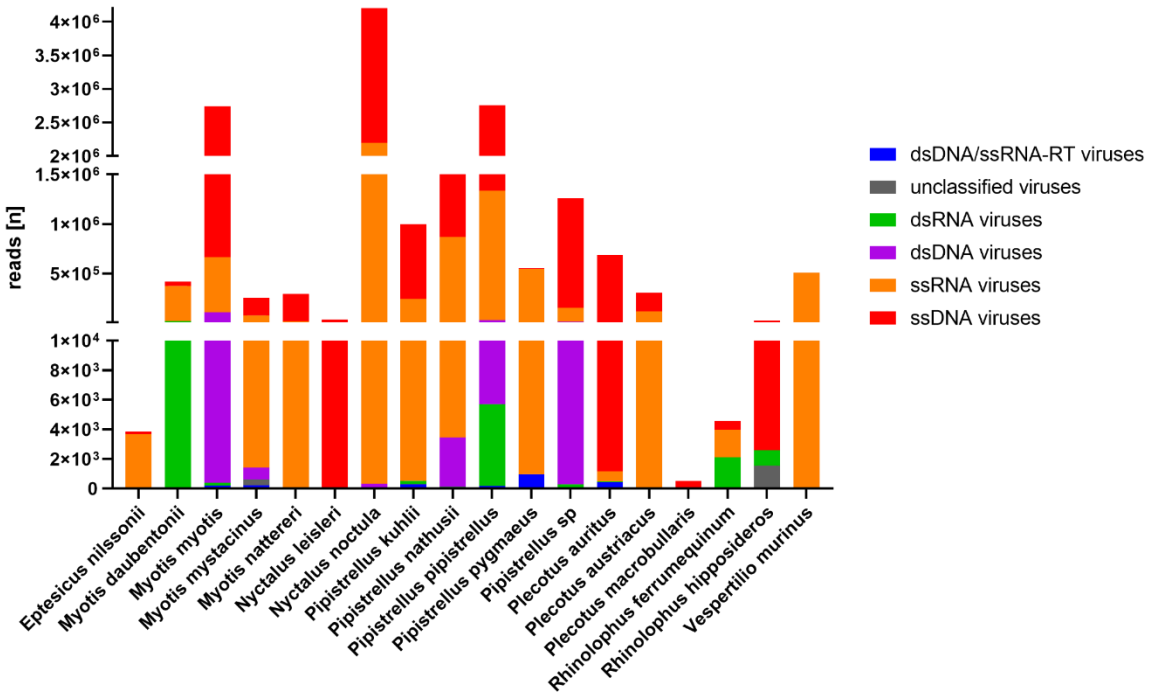
Table 2. Overview of reads assembled to different virus families categorized by sample type.

	Sample type			
Virus family	**Feces pool**	**Feces colony**	**Intestine pool**	**Tissue pool**
Vertebrate				
Adenoviridae	1.4×10^4	0	2.5×10^4	3.7×10^3
Astroviridae	1.3×10^4	0	3	0
Caliciviridae	2×10^3	0	1.9×10^3	0
Circoviridae	970	1.4×10^6	1.1×10^3	1.8×10^3
Coronaviridae	3.6×10^4	1.7×10^4	377	0
Genomoviridae	207	6×10^4	69	17
Hepeviridae	1.3×10^4			
Nairoviridae	0	0	0	1×10^4
Parvoviridae	2.2×10^3	2.6×10^4	4.1×10^3	3×10^3
Peribunyaviridae	0	353	0	0
Picobirnaviridae	0	0	99	0
Picornaviridae	0	0	2	0
Polyomaviridae	0	386	17	1×10^3
Reoviridae	1.8×10^4	2.5×10^3	8.7×10^3	273
Retroviridae	6	0	87	2.2×10^3
Unclassified	66	620	47	3
Total number of reads Vertebrate families	1×10^5	1.5×10^6	3.8×10^4	2.2×10^4
Non-vertebrate				
Alphaflexiviridae	0	1.5×10^3	0	0
Betaflexviridae	0	51	0	0
Birnaviridae	0	799	0	0
Circovirdiae	593	123	5	0
Dicistroviridae	0	4.4×10^4	1.4×10^5	380
Endornaviridae	0	0	23	0
Genomoviridae	21	1.6×10^4	0	0
Iflaviviridae	4.8×10^6	8.7×10^4	6.4×10^5	3.9×10^4
Iridoviridae	0	1.1×10^5	0	0
Nodaviridae	0	330	0	0
Partitiviridae	1	0	0	0
Parvoviridae	5×10^6	5.8×10^5	2.4×10^6	1.7×10^5
Polycipiviridae	83	2.6×10^4	9.6×10^5	1.5×10^3
Rhabdoviridae	0	0	0	100
Secoviridae	0	0	0	13
Solemoviridae	0	7.6×10^4	7	0
Tombusviridae	33	0	0	0
Tymoviridae	0	7.8×10^3	0	0
Unclassified	25	1.9×10^3	6×10^3	6
Virgaviridae	653	0	114	48
Total number of reads Non-vertebrate families	9.8×10^6	9.6×10^5	4.2×10^6	2.1×10^5
Total number of reads all virus families	$\mathbf{9.9 \times 10^6}$	$\mathbf{2.5 \times 10^6}$	$\mathbf{4.2 \times 10^6}$	$\mathbf{2.3 \times 10^5}$

Abundance of virus genomes

Reads were assembled to the inhouse database containing full length viral genomes. Virus families from all virus genome classes were detected. The genomes of 19 families of ssRNA viruses were detected of which 7 infect vertebrates, 5 insects and 7 plants. Most abundant were the ssDNA viruses with 9.7 x 10^6 assembled reads (57.7% of all assembled reads) detected in 13 bat species (Fig 4). In the remaining five bats species viruses with ssRNA genomes were the most abundant.

Fig 4. Distribution of viral reads divided into dsDNA viruses, ssDNA viruses, dsRNA viruses, ssRNA (+ssRNA and -ssRNA viruses), dsDNA/ssRNA-RT viruses, and unclassified viruses. Because of the large variations in read numbers, the legend was divided into three parts to facilitate visualization of all virus genome classes in one graph. Part one from 0 to 10^4 reads, part two from 10^4 to 1.5 x 10^5 reads, and part three from 2 x 10^6 to 4.2 x 10^6 reads.



Selected viruses of vertebrates

Viruses from 6 different virus families were studied in more detail i.e., coronaviruses (CoV), adenoviruses, rotaviruses A and H (RVA and RVH), parvoviruses (PV) and hepeviruses due to their potential zoonotic impact, circoviruses because they were the most abundant among the viruses of vertebrates detected in the bat samples.

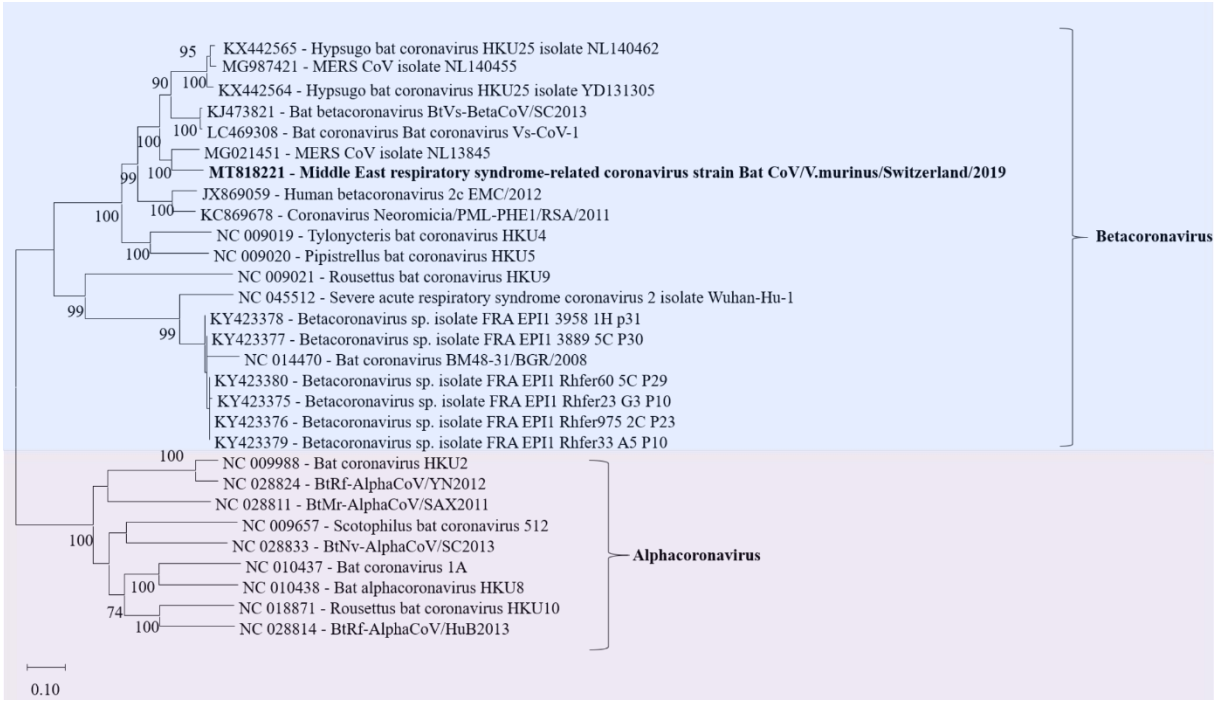
Coronaviridae

Coronaviridae are enveloped positive sensed ssRNA viruses with a virion size of 120-160nm for spherical and virion size of 75-90 nm x 170-200 nm for bacilliform morphology. The coronavirus genome consists of a single, linear genomic positive sensed RNA of 26 to 32 kb [77]. In our study 17/174 (9.8%) sample pools had reads assembled to *Coronaviridae*. In 13 ground stool pools of *Myotis myotis* and of a *Vespertilio murinus* colony, in two fecal pools of *Nyctalus noctula*, and in one intestine pool of *Pipistrellus pipistrellus*, numerous contigs between 614 and 20’189 nucleotides with high similarity (>87%) to different bat coronavirus genomes, mainly alphacoronaviruses, were assembled.

Additionally, in one ground stool sample of a *Vespertilio murinus* colony a contig of a bat coronavirus (GenBank acc. number MT818221) with a length of 20’189 bp had 86% nt similarity to a Middle East respiratory syndrome-related coronavirus (MERS-CoV) genome from China (GenBank acc. number MG021451) (Fig 5). The contig covered 3 ORF’s, including ORF1ab, ORF1a, and the ORF coding for the spike protein (S1Table).

Additionally, Sanger sequencing of a 251 bp PCR (Pancorona RT-PCR) [70] product from the polymerase gene generated a sequence of 158 nt with 100% similarity to the contig generated by de novo assembly (GenBank acc. number MT818221).

Fig 5. Phylogenetic analysis of the RNA dependent RNA polymerase gene of the coronaviruses. The sequence obtained in this study (GenBank acc. number MT818221) is shown in bold. Alphacoronaviruses are marked with a purple shaded background and betacoronaviruses with a blue shaded background. Sequences were aligned using Muscle. For phylogenetic analysis, the Maximum likelihood tree with 1'000 bootstraps was used. Only values ≥ 70% are displayed.

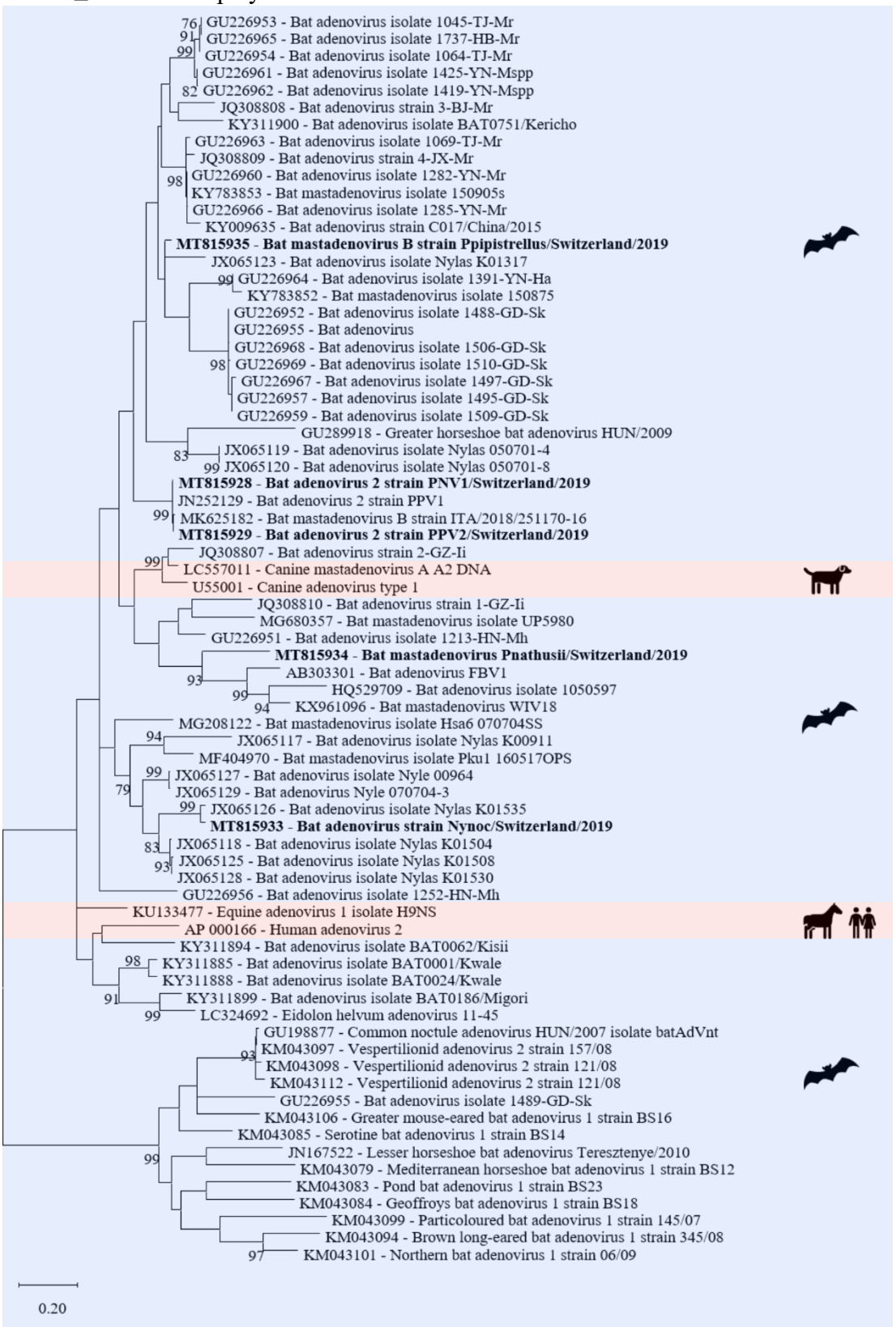


Adenoviridae

Adenoviridae are non-enveloped dsDNA viruses with an icosahedral morphology and a virion size of 70-90 nm. The genome consists of a single segment of a linear ds DNA of 26 to 48 kbp length [77]. Of the five genera of *Adenoviridae* only the genus mastadenovirus was detected in a fecal pool of *Pipistrellus nathusii*, a fecal pool of *Nyctalus noctula*, an intestine pool of *Pipistrellus pipistrellus*, an intestine pool of *Pipistrellus nathusii*, and a combined liver/spleen pool of *Pipistrellus pipistrellus*. Six assembled contigs (lengths between 2'184 and 14'493 bp) had 83 to 99% nt similarity to the genome of a bat adenovirus 2 (GenBank acc. number JN252129) (S1 Table, Fig 6).

One contig with a length of 1'673 bp had 97% nt similarity to the DNA polymerase gene of a bat adenovirus (GenBank acc. number JX065126). Three assembled contigs had 71 to 83% nt similarity to the genomes of two bat mastadenoviruses (GenBank acc. number KX961096; MK625182) (S1 Table, Fig 6). Additionally, Sanger sequencing of a PCR (Panadeno PCR) [71] product of the DNA polymerase gene generated sequences between 247-252 nt with a similarity between 79 and 100% to the contigs generated by de novo assembly of the sequencing reads.

Fig 6. Phylogenetic analysis of the adenovirus DNA polymerase gene. The sequences obtained in this study (GenBank acc. numbers MT8159528-29, MT815933-35) are shown in bold. Sequences of non-bat associated viruses are marked with a red background and bat associated viruses with a blue background. The pictograms on the right side represent the species in which the virus was detected. Sequences were aligned using Muscle. For phylogenetic analysis, the Maximum likelihood tree with 1'000 bootstraps was used. Only values ≥ 70% are displayed.



Reoviridae

Reoviridae are non-enveloped dsRNA viruses with an icosahedral morphology and a virion size of 60-85 nm. The genome consists of 9 to 12 segments with a total genome size of 19 to 32 kbp. These viruses can infect vertebrate and non-vertebrate hosts [77]. In the bat samples RVA and RVH, which belong to genus rotavirus, have been detected. [78-80].

In three samples (two fecal pools, one intestine pool) of *Myotis daubentonii* 22 contigs were assembled to RVH each covering one to two segments, i.e. NS1 to NS5 and VP1 to VP4 and VP6 (S1 Table and Figs 7 and 8). Three contigs had a nt similarity between 92 and 93% to the NSP5 segment of a porcine RVH (GenBank acc. number KT962037) from South Africa (Fig 8). Additionally, Sanger sequencing of a 666 bp PCR (Rotavirus RT-PCR) product from the NSP5 segment generated sequences of 530-543 nt with 99 to 100% similarity to the contigs generated by de novo assembly of the sequencing reads.

Fig 7. Phylogenetic analysis of specific regions of the rotavirus H genome. The sequences obtained in this study (GenBank acc. numbers MT815961-69) are shown in bold. Sequences of non-bat associated viruses are marked with a red background and bat associated viruses with a blue background. The pictograms on the right side represent the species in which the virus was detected. Sequences were aligned using Muscle. For phylogenetic analysis, the Maximum likelihood tree with 1'000 bootstraps was used. Only values ≥ 70% are displayed.

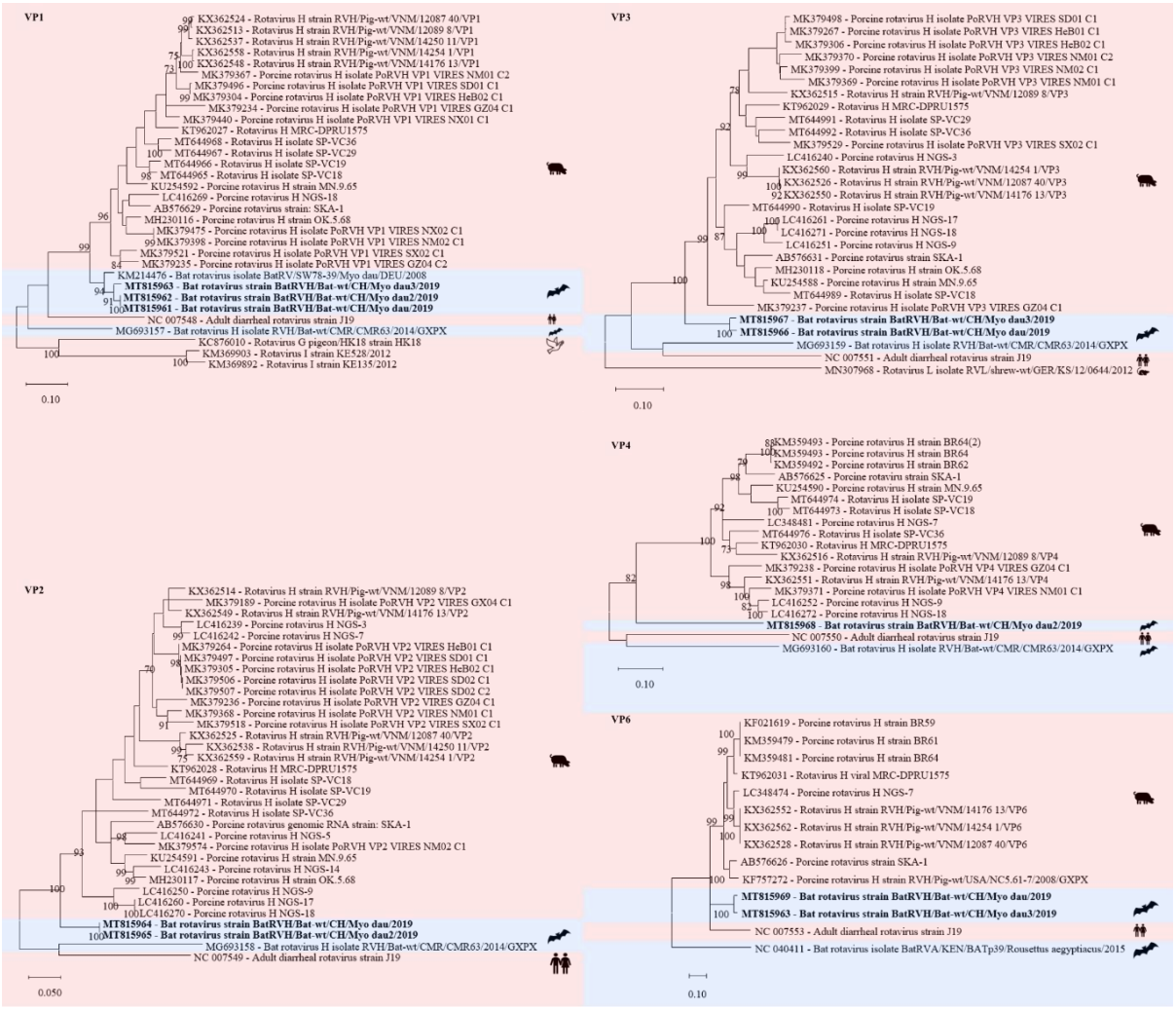
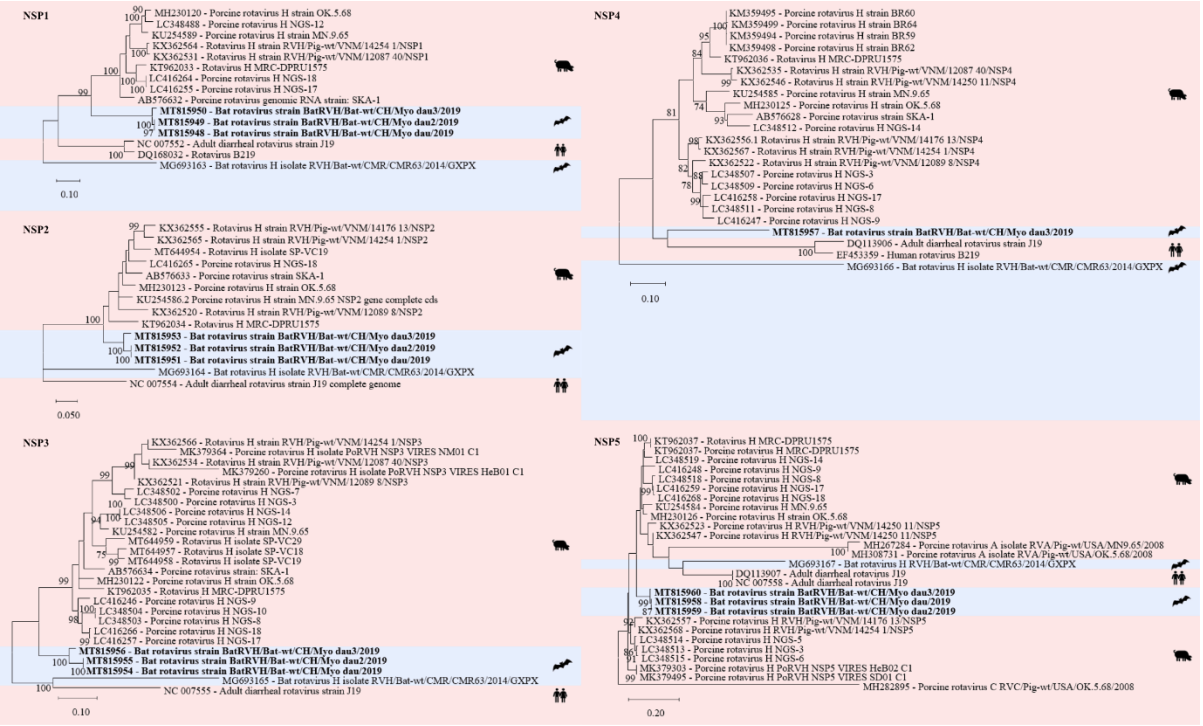
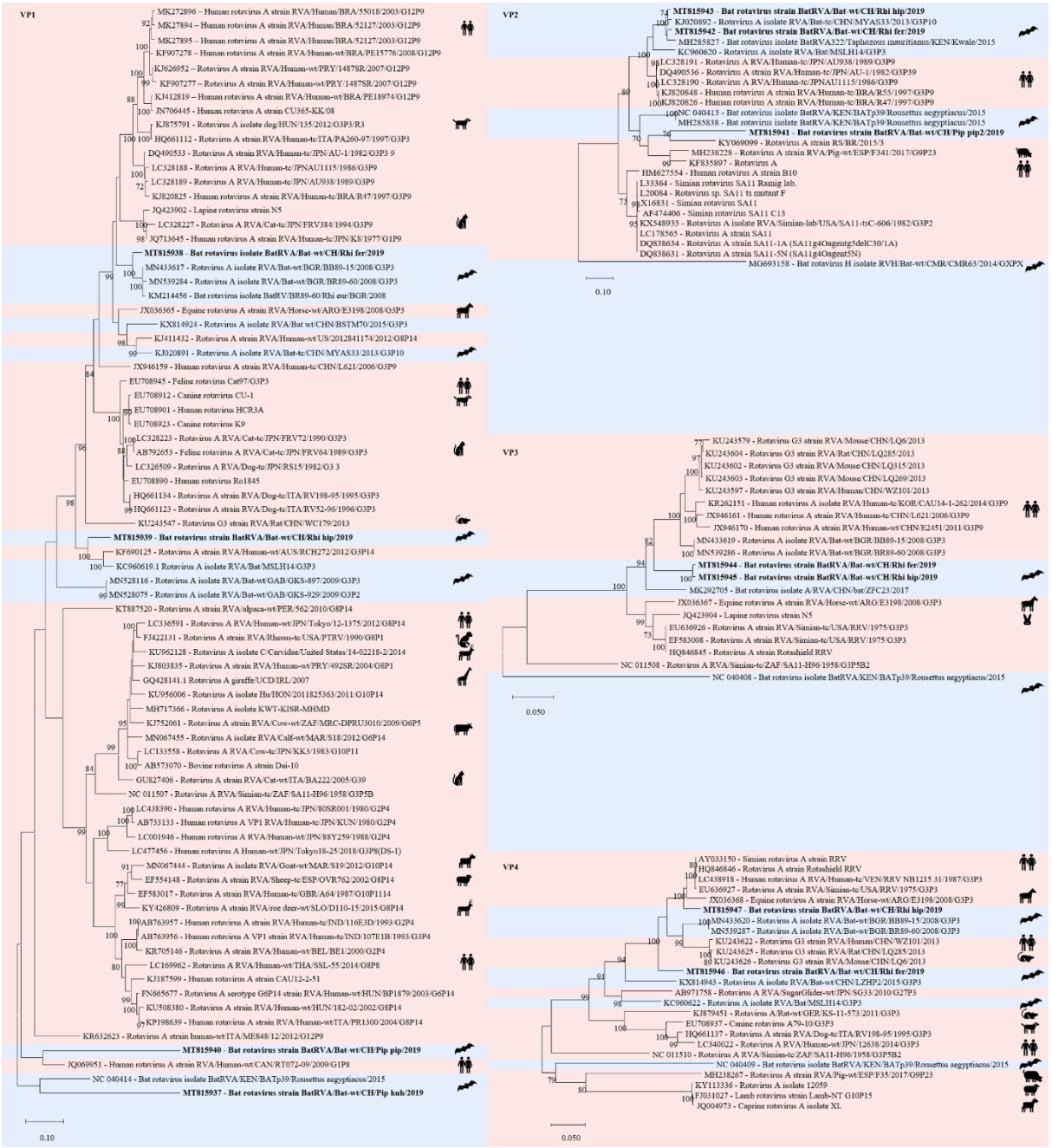


Fig 8. Phylogenetic analysis of specific regions of the rotavirus H genome. The sequences obtained in this study (GenBank acc. numbers MT815948-60) are shown in bold. Sequences of non-bat associated viruses are marked with a red background and bat associated viruses with a blue background. The pictograms on the right side represent the species in which the virus was detected. Sequences were aligned using Muscle. For phylogenetic analysis, the Maximum likelihood tree with 1'000 bootstraps was used. Only values ≥ 70% are displayed.



Furthermore, in five samples 11 contigs were assemble to RVA, each covering one to two segments of VP1 to VP4 (Table S1). The samples included a ground stool sample of a *Rhinolophus hipposiderus* colony and a *Rhinolophus ferrumequinum* colony, two intestine pools of *Pipistrellus pipistrellus* and one combined liver/spleen pool of *Pipistrellus kuhlii*. Three contigs (2'8328 bp, 1'617bp and 2'687 bp) were 96% or 73% similar to a bat RVA VP2 segment (GenBank acc. number KJ020892) or a human RVA VP2 segment (GenBank acc. number KF835897), respectively. Four contigs had 74 to 95% nt similarities to different RVA VP1 segments from bats, humans, and pigs (GenBank acc. number MN433617; MH238214; KF690125; EF583033) (Fig 9).

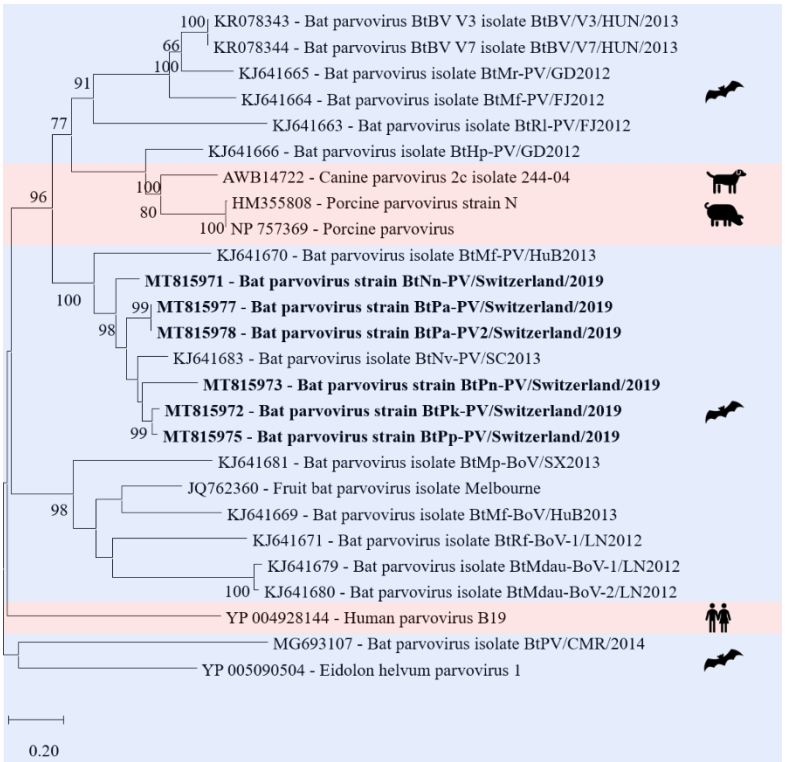
Fig 9. Phylogenetic analysis of specific regions of the rotavirus A genome. The sequences obtained in this study (GenBank acc. numbers MT815937-47) are shown in bold. Sequences of non-bat associated viruses are marked with a red background and bat associated viruses with a blue background. The pictograms on the right side represent the species in which the virus was detected. Sequences were aligned using Muscle. For phylogenetic analysis, the Maximum likelihood tree with 1'000 bootstraps was used. Only values ≥ 70% are displayed.



Parvoviridae

Parvoviridae are non-enveloped viruses with a ssDNA genome of either positive or negative polarity. The virion has an icosahedral morphology and a size of 21-26 nm. The genome consists of one linear DNA segment of 4 to 6.3 kb [77]. In total, nine contigs with lengths between 746 and 2'908 nt from different samples and bat species were assembled to one bat PV genome (GenBank acc. number KJ641683) with nt similarities between 74 and 85% (Fig 10). The contigs covered the ORFs coding for the nonstructural protein 1 (NS1) and viral protein 1 (VP1) (S1 Table). The nine contigs originated from an intestine pool of *Pipistrellus pipistrellus*, an intestine pool of *Plecotus auritus*, a fecal pool of *Nyctalus noctula*, two fecal pools of *Pipistrellus nathusii*, a combined liver/spleen pool of *Pipistrellus kuhlii*, and a combined liver/spleen pool of *Plecotus auritus*.

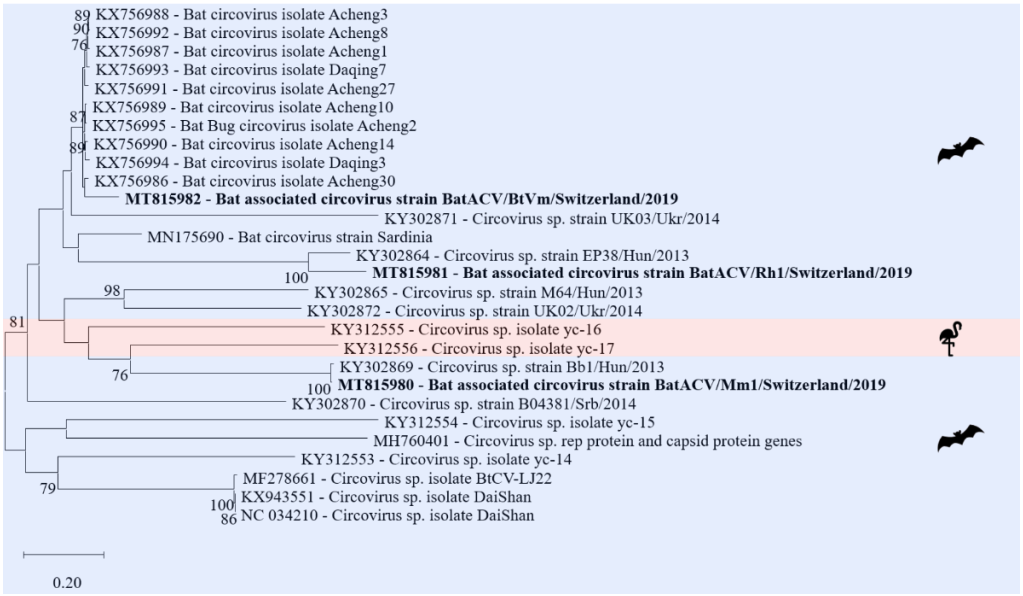
Fig 10. Phylogenetic analysis of the NS1 region of parvoviruses. The sequences obtained in this study (GenBank acc. numbers MT815971-73, MT815975, 77, 78) are shown in bold. Sequences of non-bat associated viruses are marked with a red background and bat associated viruses with a blue background. The pictograms on the right side represent the species in which the virus was detected. Sequences were aligned using Muscle. For phylogenetic analysis, the Maximum likelihood tree with 1'000 bootstraps was used. Only values ≥ 70% are displayed.



Circoviridae

Circoviridae was the most abundant vertebrate virus family in this study, with 84 % of all reads assembled to vertebrate viruses. *Circoviridae* are small non-enveloped viruses with a ssDNA genome of either positive or negative polarity. The virion has an icosahedral morphology and a size of 12-27 nm. The genome is a circular DNA with a length of 1.7-2.3 kb. These viruses infect mainly vertebrate hosts [77]. Three samples revealed full genomes, including the two ORFs coding for the replicase and the capsid protein (S1 Table) of bat-associated circoviruses. In one fecal sample of *Vespertilio murinus* a contig with a length of 2’134 nucleotides and 90% nt similarity to a bat circovirus (GenBank acc. number KX756991) from China was assembled (Fig 11). Two contigs with lengths of 1’641 bp and 1’668 bp, originating from a brain sample of a *Myotis myotis* bat, a ground stool sample of a *Myotis myotis* colony and a ground stool sample of a *Rhinolophus hipposideros* colony, with 99% nt similarity to two different Circovirus sp. (GenBank acc. number KY302869; KY302864) from Hungary were assembled (Fig 11).

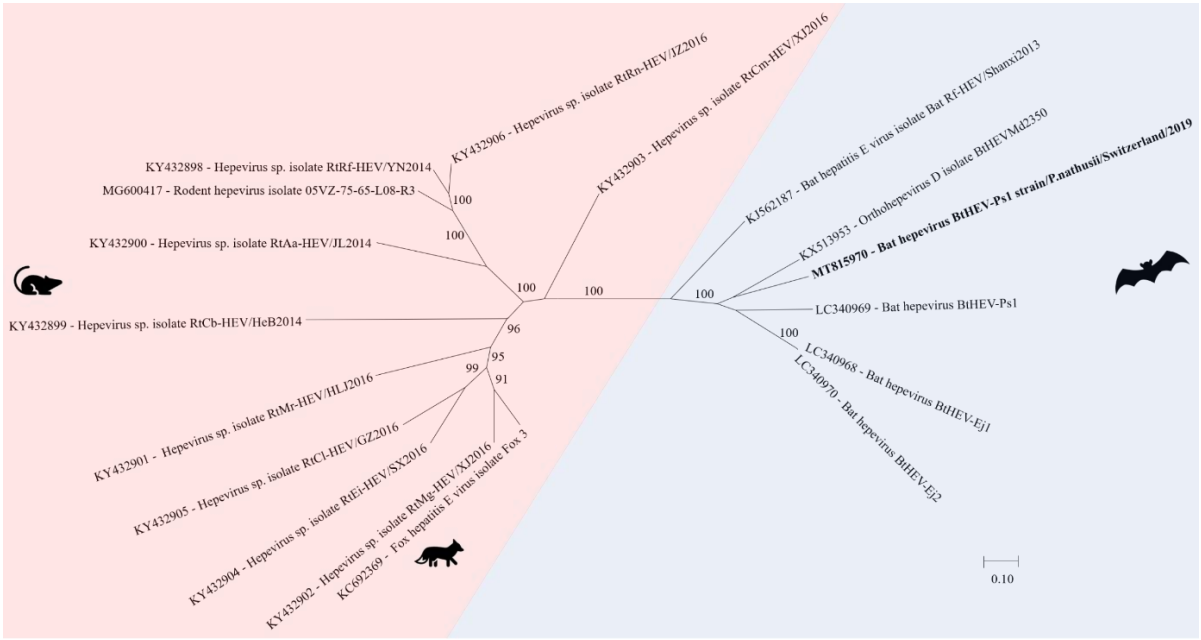
Fig 11. Phylogenetic analysis of the full genome of circoviruses. The sequences obtained in this study (GenBank acc. numbers MT815980-82) are shown in bold. Sequences of non-bat associated viruses are marked with a red background and bat associated viruses with a blue background. The pictograms on the right side represent the species in which the virus was detected. Sequences were aligned using Muscle. For phylogenetic analysis, the Maximum likelihood tree with 1’000 bootstraps was used. Only values ≥ 70% are displayed.



Hepeviridae

Hepeviridae are small non-enveloped positive sensed ssRNA viruses. They have an icosahedral morphology with a size of 27-34 nm. The genome consists of a single, linear RNA of 6.6 to 7.2 kb. *Hepeviridae* have so far been detected only in vertebrate hosts. [77] In a fecal pool of *Pipistrellus nathusii*, an orthohepevirus D contig with a length of 6’647 nt was assembled with 75% nt similarity to a bat hepevirus genome from China (GenBank acc. number KX513953). The contig covered two ORF’s coding for the nonstructural polyprotein and the capsid protein (S1 Table). In the phylogenetic analysis the separate clade of bat hepevirus is shown (Fig 12).

Fig 12. Phylogenetic analysis of the capsid protein of hepeviruses. The sequence obtained in this study (GenBank acc. numbers MT815970) is shown in bold. Sequences of non-bat associated viruses are marked with a red background and bat associated viruses with a blue background. The pictograms on the right side represent the species in which the virus was detected. Sequences were aligned using Muscle. For phylogenetic analysis, the Maximum likelihood tree with 1’000 bootstraps was used. Only values ≥ 70% are displayed.



Discussion

This study included samples from 7’291 bats of 18 different species. Specifically, organ or fecal samples from 245 individual animals and ground stool samples from 36 different colonies with approximately 7’046 individual animals from 8 different bat species were analyzed by NGS. This metagenomic analysis revealed the assemblies to the genomes of a total of 39 different virus families including 16 virus families of vertebrates (Fig 2A and 2B). Similar viral metagenomic studies of bats from Singapore, China, and Myanmar have yielded comparable virus diversities [6, 64, 81, 82]. Interestingly, the data obtained in a study conducted in France is strikingly different. While the number of different virus families of vertebrates detected was much smaller (8), it included many virus families that were not detected in our study, such as *Bunyaviridae*, *Flaviviridae*, *Herpesviridae*, and *Orthomyxoviridae* [5]. These differences may be due to the different populations sampled in that study (bats with behavioral changes and close human contact) compared to our study (mainly healthy bats) as well as the larger numbers of different bat species (18) and sample types (fecal samples, liver, spleen, lung, brain and intestine) investigated in our study.

The question whether native bat species harbor CoVs was of special interest in this study. Such virus genomes were indeed detected and belonged to either the alpha-or betacoronaviruses while no gamma- or deltacoronaviruses were detected in the samples. This finding was consistent with the results of previous studies in other geographical locations [34, 42, 50, 55, 83-85] and the common knowledge about host preferences of the different coronaviruses; alpha- and betacoronaviruses mainly infect mammals including humans while the gamma- and deltacoronaviruses infect birds [6, 86]. However, so far none of the coronaviruses detected in European bats were 100% identical to human pathogenic ones such as SARS-CoV and 2 or MERS-CoV [34]. Nevertheless, closely related CoVs have been detected i.e., in Italy, where the genomes of two viruses related to MERS-CoV, 80% nt

similarity [85], have been detected in *Pipistrellus pipistrellus* and *Hypsugo savii*. Similarly, in a ground stool sample of a *Vespertilio murinus* colony sampled in our study, a contig of 20 kb with a nt similarity of 86% to a MERS-like CoV genome from China was assembled [87].

Rhabdovirus sequences were not identified in our study, although rabies lyssaviruses have previously been detected in *Myotis daubentonii* bats in Switzerland, albeit at low prevalence (0.36%) [40]. Rhabdoviruses, especially viruses from the genus Lyssavirus, are zoonotic viruses. In Europe, several lyssaviruses were detected in bats including the European bat lyssavirus 1 and 2 (EBLV-1/2). It is known that bats can transmit rabies/lyssaviruses to humans by biting and scratching [34]. In the present study neither EBLV-1 nor EBLV-2 were detected. In Europe *Eptesicus serotinus* and *Myotis daubentonii*, both endemic also in Switzerland, are known to be the main hosts of EBLV-1 or 2, respectively [7, 34]. In this study only samples of *Myotis daubentonii* were collected.

In Switzerland five bat species, *Nyctalus leisleri, N. noctula, N. lasiopterus, Pipistrellus nathusii and Vespertilio murinu*s, migrate several hundred kilometers between summer and winter quarter [7]. Migration habits may lead to harboring more viruses due to broader contact with various surroundings e.g. other bat populations, other living areas, and insects. However, in this study, only in two migrating species i.e., *Pipistrellus nathusii* and *Vespertilio murinus,* the number of different virus families detected was higher than the average 9.6 virus families per bat species, with 16 and 10, respectively. The observation that the virome of migrating bats is not more diverse than that of non-migrating bats has been made in previous studies [16, 17, 88, 89].

In the present study, 90% of all assembled reads belonged to virus families of non-vertebrates. This observation is not consistent with previous reports where vertebrate viruses clearly dominated the virome [5, 6, 61, 62, 64, 65, 90]. This difference may be explained by the

different sample material used (only feces or only tissue samples), in our case a combination of feces and organs [5, 6, 61, 62, 64, 65, 90]. The virome of bat guano from Texas and California, and from Singapore consisted of mainly non-vertebrate viruses, particularly insect viruses, thereby supporting the hypothesis that dietary habits are a likely explanation for the large numbers of insect viruses detected in fecal samples [61-64, 90]. Although most of our samples were pooled organs, more reads were assembled to non-vertebrate viruses, since all sampled bat species were insectivorous, and a higher number of reads was generated in fecal pools. Another interesting observation was the high number of reads assembled to honeybee viruses in fecal pools of *Myotis myotis* colonies. Bee-associated viruses were found also in different bat species from other countries [61-63, 90]. The detected sequences were mostly related to the deformed wing virus (*Varrora destructor* virus) which causes either asymptomatic infection in western honeybees (*Apis mellifera*) or can lead to wing deformity, behavioral changes, and early mortality in other bee species [61, 91].

Adenoviruses have been detected in bats from several countries including Italy, Germany, China, and Hungary, however, the number of different adenoviruses detected in bats is small [38, 42, 92-95]. The genus *Mastadenovirus* has been thought to infect only mammals, including humans [38, 96, 97]. However, cross-species transmission has nevertheless been observed with adenoviruses, and circulation between different animal species and transmission from animals to humans is possible [38, 98, 99].

Most metagenomic studies of bats use feces as sample material. As *Parvoviridae* can be detected in high concentrations in human blood samples [100], Canuti et al. sampled EDTA-blood of two bat species and showed a relatively low prevalence of PVs in bats from West Africa and Central America bats [41]. In our study nearly a quarter of the samples had reads assembled to *Parvovirinae*, mainly bat PVs. For the emergence of new viruses, their evolutionary potential and mutation rate is of particular importance. RNA viruses are known

to mutate much more frequently than DNA viruses, however, among the DNA viruses the PVs have a relatively high mutation rate, and host switching has been observed [101, 102]. On the other hand, PVs are known to be coevolving with their host [103].

RVA and RVH have been detected in several previous bat metagenomic studies from different locations [78-80]. However, it is not known whether RV infections in bats leads to disease [79, 104]. RVs have been detected also in several other wild animals [105].

Bats are known hosts for different hepeviruses of the genus *Orthohepevirus* [106-108]. Bat hepeviruses, including the contig revealed in this study, seem to cluster with one another and generate a separate clade withing the *Orthohepeviruses*, the Orthohepevirus D [106, 107]. This indicates that bats may not serve as reservoirs of hepeviruses infecting other mammals including humans [106].

The most interesting finding in this study was an almost complete genome of a MERS-like CoV detected in a ground stool sample of a *Vespertilio murinus* colony. It would be interesting to study the zoonotic potential of this bat betacoronavirus and to monitor the colony in which it was detected over time as this would allow to assess the accumulation of mutations in the CoV genome in a natural reservoir host.

Human interaction with wildlife is one of the major contributors for emergence of zoonotic spillover and this impact should be reassessed [58]. Metagenomic analysis of ground stool samples of bat colonies represents an ideal non-invasive high throughput method to monitor the complexity of the virome. It allows also to detect viruses with zoonotic potential and to assess the potential risk for their transmission to other species including humans. This first assessment of the virome of Swiss bats revealed a large viral genome diversity and forms a platform for future in-depth studies to investigate changes in virus prevalence, virus biology, virus-host interaction, and virus emergence.

Acknowledgements

We want to thank the Bat Foundation Switzerland, the cantonal commissioners for bat protection, the care takers of bat colonies for their help with the sample collection, and Elisabeth Schraner for assistance with electron microscopy.

References

1. Frick WF, Kingston T, Flanders J. A review of the major threats and challenges to global bat conservation. Annal of the New York Academy of Sciences. 2019. Epub 2019/04/02. doi: 10.1111/nyas.14045.
2. Simmons NBaALC. *Bat Species of the World: A taxonomic and geographic database* . c2020 [cited 2020 Apr 28]. Available from: https://batnames.org/home.html.
3. Amador LI, Arévalo RLM, Almeida FC, Catalano SA, Giannini NP. Bat systematics in the light of unconstrained analyses of a comprehensive molecular supermatrix. J Mamm Evol. 2018;25(1):37-70. doi: 10.1007/s10914-016-9363-8.
4. Burgin CJ, Colella JP, Kahn PL, Upham NS. How many species of mammals are there? J Mammal 2018;99(1):1-14. doi: 10.1093/jmammal/gyx147.
5. Dacheux L, Cervantes-Gonzalez M, Guigon G, Thiberge JM, Vandenbogaert M, Maufrais C, et al. A preliminary study of viral metagenomics of French bat species in contact with humans: identification of new mammalian viruses. PLoS One. 2014;9(1):e87194. Epub 2014/01/29. doi: 10.1371/journal.pone.0087194.
6. Hu D, Zhu C, Wang Y, Ai L, Yang L, Ye F, et al. Virome analysis for identification of novel mammalian viruses in bats from Southeast China. Sci Rep. 2017;7(1):10917. Epub 2017/09/07. doi: 10.1038/s41598-017-11384-w.
7. fledermausschutz.ch [Internet]. Switzerland: Bat conservation; c2020 [cited 2020 Apr 16]. Available from: https://fledermausschutz.ch/.
8. iucn.org [Internet]. Switzerland: International Union for Conservation of Nature; c2020 [cited 2020 Apr 16]. Available from: https://www.iucn.org/content/microchiropteran-bats-global-status-survey-and-conservation-action-plan
9. batlife-europe.info [Internet]. England: BatLife Europe: Bat Conservation; c2020 [cited 2020 Apr 30]. Available from: https://www.batlife-europe.info/.
10. Teeling EC, Vernes SC, Dávalos LM, Ray DA, Gilbert MTP, Myers E, et al. Bat Biology, Genomes, and the Bat1K Project: To Generate Chromosome-Level Genomes for All Living Bat Species. Annu Rev Anim Biosci. 2018;6:23-46. Epub 2017/11/20. doi: 10.1146/annurev-animal-022516-022811.
11. Riccucci M, Lanza B. Bats and insect pest control: a review. Vespertilio. 2014;17:161-9.
12. Bumrungsri S, Lang D, Harrower C, Sripaoraya E, Kitpipit K, Racey PA. The dawn bat, Eonycteris spelaea Dobson (Chiroptera: Pteropodidae) feeds mainly on pollen of economically important food plants in Thailand. Acta Chiropterologica. 2013;15(1):95-104. doi: 10.3161/150811013x667894.
13. Lina PH. Common names of European bats: UNEP/EUROBATS Secretariat; 2016.
14. Shipley R, Wright E, Selden D, Wu G, Aegerter J, Fooks AR, et al. Bats and Viruses: Emergence of Novel Lyssaviruses and Association of Bats with Viral Zoonoses in the EU. Trop Med Infect Dis. 2019;4(1). Epub 2019/02/07. doi: 10.3390/tropicalmed4010031.

15. Griffin DR. Griffin, D. R. 1970. Migrations and homing in bats, p. 233–264. In W. A. Wimsatt (ed.), Biology of bats. Academic Press, New York, N.Y. 1970:233 - 64. doi: 10.1016/B978-0-12-758001-2.
16. Calisher CH, Childs JE, Field HE, Holmes KV, Schountz T. Bats: important reservoir hosts of emerging viruses. Clin Microbiol Rev. 2006;19(3):531-45. doi: 10.1128/CMR.00017-06.
17. Brosset A. The migrations of Pipistrellus nathusii, in France. Possible implication on the spreading of rabies. Mammalia. 1990. doi: 10.1515/mamm-1990-0205.
18. Mollentze N, Streicker DG. Viral zoonotic risk is homogenous among taxonomic orders of mammalian and avian reservoir hosts. Proceedings of the National Academy of Sciences. 2020;117(17):9423-30. doi: 10.1073/pnas.1919176117.
19. Olival KJ, Epstein JH, Wang L-F, Field HE, Daszak P. Are bats exceptional viral reservoirs. New directions in conservation medicine: Applied cases of ecological health. Bats and Viruses. 2012:195-212. doi: 10.1002/9781118818824.ch11.
20. Smith I, Wang LF. Bats and their virome: an important source of emerging viruses capable of infecting humans. Curr Opin Virol. 2013;3(1):84-91. Epub 2012/12/21. doi: 10.1016/j.coviro.2012.11.006.
21. Wilkinson GS, South JM. Life history, ecology and longevity in bats. Aging cell. 2002;1(2):124-31. doi: 10.1046/j.1474-9728.2002.00020.x.
22. Wilkinson GS, Adams DM. Recurrent evolution of extreme longevity in bats. Biol Lett. 2019;15(4):20180860. doi: 10.1098/rsbl.2018.0860.
23. Podlutsky AJ, Khritankov AM, Ovodov ND, Austad SN. A new field record for bat longevity. J Gerontol A Biol Sci Med Sci. 2005;60(11):1366-8. doi: 10.1093/gerona/60.11.1366.
24. Zhang G, Cowled C, Shi Z, Huang Z, Bishop-Lilly KA, Fang X, et al. Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. Science. 2013;339(6118):456-60. Epub 2012/12/20. doi: 10.1126/science.1230835.
25. Baker ML, Schountz T, Wang LF. Antiviral immune responses of bats: a review. Zoonoses Public Health. 2013;60(1):104-16. Epub 2012/08/01. doi: 10.1111/j.1863-2378.2012.01528.x.
26. O'Shea TJ, Cryan PM, Cunningham AA, Fooks AR, Hayman DT, Luis AD, et al. Bat flight and zoonotic viruses. Emerg Infect Dis. 2014;20(5):741-5. doi: 10.3201/eid2005.130539.
27. Sulkin SE, Allen R. Virus infections in bats. Monogr Virol. 1974;8(0):1-103. doi: 10.1159/000395514.
28. Lyman CP. Lyman, C. P. 1970. Thermoregulation and metabolism in bats, p. 301–330. In W. A. Wimsatt (ed.), Biology of bats. Academic Press, New York, N.Y.1970.
29. Banerjee A, Misra V, Schountz T, Baker ML. Tools to study pathogen-host interactions in bats. Virus Res. 2018;248:5-12. Epub 2018/02/15. doi: 10.1016/j.virusres.2018.02.013.
30. Halpin K, Hyatt AD, Fogarty R, Middleton D, Bingham J, Epstein JH, et al. Pteropid bats are confirmed as the reservoir hosts of henipaviruses: a comprehensive experimental study of virus transmission. Am J Trop Med Hyg. 2011;85(5):946-51. doi: 10.4269/ajtmh.2011.10-0567.
31. Schuh AJ, Amman BR, Jones ME, Sealy TK, Uebelhoer LS, Spengler JR, et al. Modelling filovirus maintenance in nature by experimental transmission of Marburg virus between Egyptian rousette bats. Nat Commun. 2017;8(1):1-10. doi: 10.1038/ncomms14446.
32. Kuno G. Persistence of arboviruses and antiviral antibodies in vertebrate hosts: its occurrence and impacts. Rev Med Virol. 2001;11(3):165-90. doi: 10.1002/rmv.314.

33. Banerjee A, Rapin N, Bollinger T, Misra V. Lack of inflammatory gene expression in bats: a unique role for a transcription repressor. Sci Rep. 2017;7(1):2232. Epub 2017/05/22. doi: 10.1038/s41598-017-01513-w.
34. Kohl C, Kurth A. European bats as carriers of viruses with zoonotic potential. Viruses. 2014;6(8):3110-28. Epub 2014/08/13. doi: 10.3390/v6083110.
35. Sendow I, Field HE, Curran J. Henipavirus in Pteropus vampyrus bats, Indonesia. Emerg Infect Dis. 2006;12(4):711. doi: 10.3201/eid1204.051181.
36. Reynes J-M, Counor D, Ong S, Faure C, Seng V, Molia S, et al. Nipah virus in Lyle's flying foxes, Cambodia. Emerg Infect Dis. 2005;11(7):1042. doi: 10.3201/eid1107.041350.
37. Philbey AW, Kirkland PD, Ross AD, Davis RJ, Gleeson AB, Love RJ, et al. An apparently new virus (family Paramyxoviridae) infectious for pigs, humans, and fruit bats. Emerg Infect Dis. 1998;4(2):269. doi: 10.3201/eid0402.980214.
38. Diakoudi G, Lanave G, Moreno A, Chiapponi C, Sozzi E, Prosperi A, et al. Surveillance for Adenoviruses in Bats in Italy. Viruses. 2019;11(6). Epub 2019/06/06. doi: 10.3390/v11060523.
39. Monath TP. Ecology of Marburg and Ebola viruses: speculations and directions for future research. J Infect Dis. 1999;179(Supplement_1):S127-S38. doi: 10.1086/514281.
40. Megali A, Yannic G, Zahno ML, Brügger D, Bertoni G, Christe P, et al. Surveillance for European bat lyssavirus in Swiss bats. Arch Virol. 2010;155(10):1655-62. Epub 2010/08/28. doi: 10.1007/s00705-010-0750-9.
41. Canuti M, Eis-Huebinger AM, Deijs M, de Vries M, Drexler JF, Oppong SK, et al. Two novel parvoviruses in frugivorous New and Old World bats. PLoS One. 2011;6(12): e29140. doi: 10.1371/journal.pone.0029140.
42. Han HJ, Wen HL, Zhao L, Liu JW, Luo LM, Zhou CM, et al. Novel coronaviruses, astroviruses, adenoviruses and circoviruses in insectivorous bats from northern China. Zoonoses Public Health. 2017;64(8):636-46. doi: 10.1111/zph.12358.
43. Male MF, Kraberger S, Stainton D, Kami V, Varsani A. Cycloviruses, gemycircularviruses and other novel replication-associated protein encoding circular viruses in Pacific flying fox (Pteropus tonganus) faeces. Infect Genet Evol. 2016;39:279-92. doi: 10.1016/j.meegid.2016.02.009.
44. Plowright RK, Field HE, Smith C, Divljan A, Palmer C, Tabor G, et al. Reproduction and nutritional stress are risk factors for Hendra virus infection in little red flying foxes (Pteropus scapulatus). Proc. Royal Soc. B. 2008;275(1636):861-9. doi: 10.1098/rspb.2007.1260.
45. Leroy E, Gonzalez J, Pourrut X. Ebolavirus and other filoviruses. Wildlife and Emerging Zoonotic Diseases: The biology, circumstances and consequences of cross-species transmission: Curr Top Microbiol Immunol. Springer; 2007; p. 363-87. doi: 10.1007/978-3-540-70962-6_15.
46. Wimsatt WA. Biology of bats Chapter 1 - Bat Origins and Evolution. New York, N.Y.: Elsevier Inc. All; 1970. 1 - 64 p.
47. Hayman DT. Bats as Viral Reservoirs. Annu Rev Virol. 2016;3(1):77-99. Epub 2016/08/22 . doi: 10.1146/annurev-virology-110615-042203.
48. Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, et al. Bats are natural reservoirs of SARS-like coronaviruses. Science. 2005;310(5748):676-9. Epub 2005/09/29. doi: 10.1126/science.1118391.
49. Wang LF, Anderson DE. Viruses in bats and potential spillover to animals and humans. Curr Opin Virol. 2019;34:79-89. Epub 2019/01/18. doi: 10.1016/j.coviro.2018.12.007.
50. Fan Y, Zhao K, Shi ZL, Zhou P. Bat Coronaviruses in China. Viruses. 2019;11(3). Epub 2019/03/02. doi: 10.3390/v11030210.

51. Chen L, Liu B, Yang J, Jin Q. DBatVir: the database of bat-associated viruses. Database (Oxford). 2014;2014:bau021. Epub 2014/03/18. doi: 10.1093/database/bau021.
52. mgc.ac.cn/DBatVir/ [Internet]. China: DBatVir Database of bat-associated viruses.; c2020 [cited 2020 Apr 20]. Available from: http://www.mgc.ac.cn/DBatVir/.
53. Field H, Young P, Yob JM, Mills J, Hall L, Mackenzie J. The natural history of Hendra and Nipah viruses. Microbes Infect. 2001;3(4):307-14. doi: 10.1016/s1286-4579(01)01384-3.
54. Zaki AM, Van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med. 2012;367(19):1814-20. doi: 10.1056/nejmx130029.
55. Hu B, Ge X, Wang L-F, Shi Z. Bat origin of human coronaviruses. Virol J. 2015;12(1):221. doi: 10.1186/s12985-015-0422-1.
56. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-3. Epub 2020/02/03. doi: 10.1038/s41586-020-2012-7.
57. Paraskevis D, Kostaki EG, Magiorkinis G, Panayiotakopoulos G, Sourvinos G, Tsiodras S. Full-genome evolutionary analysis of the novel corona virus (2019-nCoV) rejects the hypothesis of emergence as a result of a recent recombination event. Infect Genet Evol. 2020;79:104212. doi: 10.1101/2020.01.26.920249.
58. Frutos R, Serra-Cobo J, Chen T, Devaux CA. COVID-19: Time to exonerate the pangolin from the transmission of SARS-CoV-2 to humans. Infect Genet Evol. 2020;84:104493. doi: 10.1016/j.meegid.2020.104493.
59. Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The proximal origin of SARS-CoV-2. Nat Med. 2020;26(4):450-2. doi: 10.1038/s41591-020-0820-9.
60. Tang X, Wu C, Li X, Song Y, Yao X, Wu X, et al. On the origin and continuing evolution of SARS-CoV-2. Natl Sci Rev. 2020; 7(6):1012-23. doi: 10.1093/nsr/nwaa036.
61. Ge X, Li Y, Yang X, Zhang H, Zhou P, Zhang Y, et al. Metagenomic analysis of viruses from bat fecal samples reveals many novel viruses in insectivorous bats in China. J Virol. 2012;86(8):4620-30. Epub 2012/02/15. doi: 10.1128/JVI.06671-11.
62. Donaldson EF, Haskew AN, Gates JE, Huynh J, Moore CJ, Frieman MB. Metagenomic analysis of the viromes of three North American bat species: viral diversity among different bat species that share a common habitat. J Virol. 2010;84(24):13004-18. Epub 2010/10/06. doi: 10.1128/JVI.01255-10.
63. Li L, Victoria JG, Wang C, Jones M, Fellers GM, Kunz TH, et al. Bat guano virome: predominance of dietary viruses from insects and plants plus novel mammalian viruses. J Virol. 2010;84(14):6955-65. Epub 2010/05/12. doi: 10.1128/JVI.00501-10.
64. He B, Li Z, Yang F, Zheng J, Feng Y, Guo H, et al. Virome profiling of bats from Myanmar by metagenomic analysis of tissue samples reveals more novel Mammalian viruses. PLoS One. 2013;8(4):e61950. Epub 2013/04/22. doi: 10.1371/journal.pone.0061950.
65. Wu Z, Ren X, Yang L, Hu Y, Yang J, He G, et al. Virome analysis for identification of novel mammalian viruses in bat species from Chinese provinces. J Virol. 2012;86(20):10999-1012. Epub 2012/08/01. doi: 10.1128/JVI.01394-12.
66. Yang F, Wang Y, Zheng W, He B, Jiang T, Li Y, et al. Metagenomic analysis of bat virome in several Chinese regions. Sheng Wu Gong Cheng Xue Bao. 2013;29(5):586-600. doi: 10.1128/msphere.00587-20.
67. Zheng XY, Qiu M, Guan WJ, Li JM, Chen SW, Cheng MJ, et al. Viral metagenomics of six bat species in close contact with humans in southern China. Arch Virol. 2018;163(1):73-88. Epub 2017/10/05. doi: 10.1007/s00705-017-3570-3.
68. Kubacki J, Flacio E, Qi W, Guidi V, Tonolla M, Fraefel C. Viral Metagenomic Analysis of Aedes albopictus Mosquitos from Southern Switzerland. Viruses. 2020;12(9):929. doi:10.3390/v12090929.

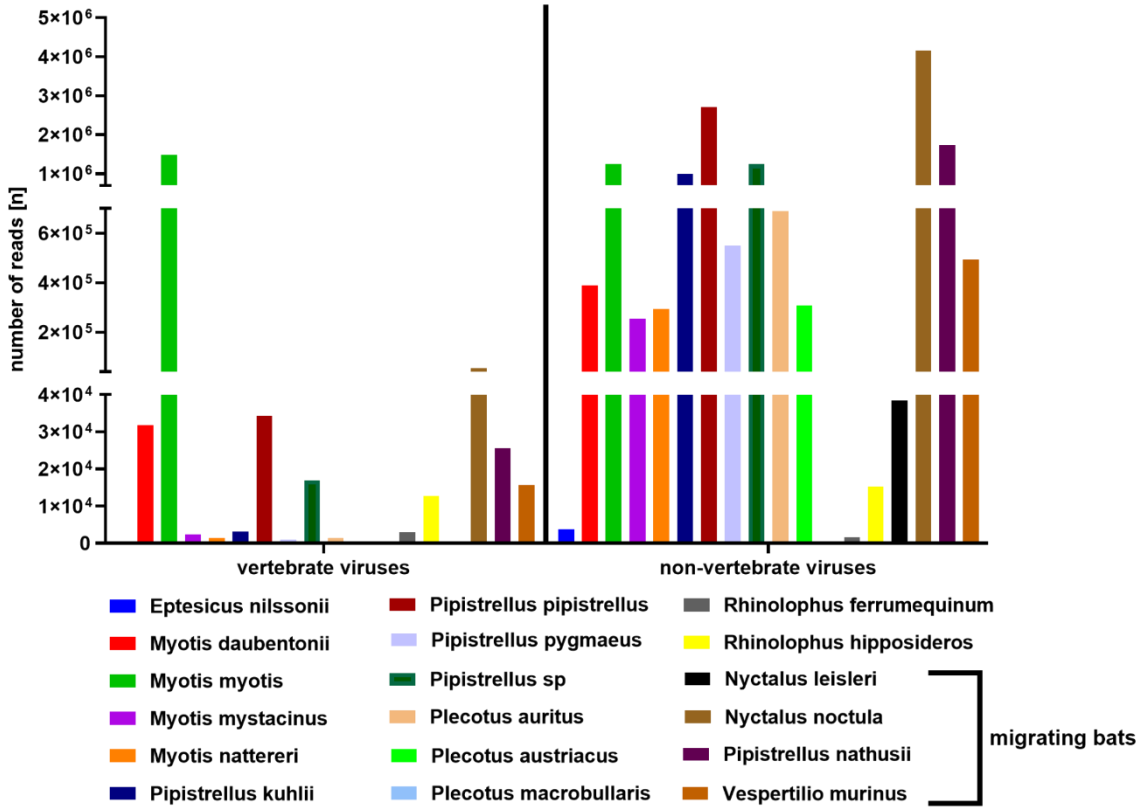
69. Bachofen C, Kubacki J, Jermini M, Giannini P, Martinetti G, Ripellino P, et al. New subcluster of HEV genotype 3 strains linked to the first confirmed Swiss case of foodborne hepatitis E infection. Case Rep. J. 2017;1(1):003. doi: 10.5167/uzh-148669.
70. Vijgen L, Moës E, Keyaerts E, Li S, Van Ranst M. A pancoronavirus RT-PCR assay for detection of all known coronaviruses. Methods Mol Biol. 2008;454:3-12. doi: 10.1007/978-1-59745-181-9_1.
71. Wellehan JF, Johnson AJ, Harrach B, Benkö M, Pessier AP, Johnson CM, et al. Detection and analysis of six lizard adenoviruses by consensus primer PCR provides further evidence of a reptilian origin for the atadenoviruses. J Virol. 2004;78(23):13366-9. doi: 10.1128/JVI.78.23.13366-13369.2004.
72. Untergasser A, Nijveen H, Rao X, Bisseling T, Geurts R, Leunissen JAM. Primer3Plus, an enhanced web interface to Primer3. Nucleic acids research. 2007;35(Web Server issue):W71-W4. Epub 2007/05/07. doi: 10.1093/nar/gkm306.
73. Li D, Liu C-M, Luo R, Sadakane K, Lam T-W. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics. 2015;31(10):1674-6. doi: 10.1093/bioinformatics/btv033.
74. NCBI, database n [Internet]. United States of America: National Center for Biotechnology Information database n; c2020 [cited 2020 Nov 01]. Available from: ftp://ftp.ncbi.nlm.nih.gov/blast/db/
75. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215(3):403-10. doi: 10.1016/S0022-2836(05)80360-2.
76. Kumar S SG, Li M, Knyaz C, and Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol. 2018;35:1547-9. doi: 10.1093/molbev/msy096.
77. King AM, Lefkowitz E, Adams MJ, Carstens EB. Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses: Elsevier; 2011.
78. Esona MD, Mijatovic-Rustempasic S, Conrardy C, Tong S, Kuzmin IV, Agwanda B, et al. Reassortant group A rotavirus from straw-colored fruit bat (Eidolon helvum). Emerg Infect Dis. 2010;16(12):1844. doi: 10.3201/eid1612.101089.
79. Yinda CK, Ghogomu SM, Conceição-Neto N, Beller L, Deboutte W, Vanhulle E, et al. Cameroonian fruit bats harbor divergent viruses, including rotavirus H, bastroviruses, and picobirnaviruses using an alternative genetic code. Virus Evol. 2018;4(1):vey008. Epub 2018/03/30. doi: 10.1093/ve/vey008.
80. Kim HK, Yoon SW, Kim DJ, Koo BS, Noh JY, Kim J, et al. Detection of Severe Acute Respiratory Syndrome-Like, Middle East Respiratory Syndrome-Like Bat Coronaviruses and Group H Rotavirus in Faeces of Korean Bats. Transbound Emerg Dis. 2016;63(4):365-72. doi: 10.1111/tbed.13651.
81. Wu Z, Yang L, Ren X, He G, Zhang J, Yang J, et al. Deciphering the bat virome catalog to better understand the ecological diversity of bat viruses and the bat origin of emerging infectious diseases. ISME J. 2016;10(3):609-20. Epub 2015/08/11. doi: 10.1038/ismej.2015.138.
82. Mendenhall IH, Wen DLH, Jayakumar J, Gunalan V, Wang L, Mauer-Stroh S, et al. Diversity and Evolution of Viral Pathogen Community in Cave Nectar Bats. Viruses. 2019;11(3). Epub 2019/03/12. doi: 10.3390/v11030250.
83. Kivistö I, Tidenberg E-M, Lilley T, Suominen K, Forbes KM, Vapalahti O, et al. First report of coronaviruses in Northern European bats. Vector Borne Zoonotic Dis. 2020;20(2):155-8. doi: 10.1089/vbz.2018.2367.
84. Bonny TS, Driver JP, Paisie T, Salemi M, Morris JG, Shender LA, et al. Detection of Alphacoronavirus vRNA in the Feces of Brazilian Free-Tailed Bats (Tadarida brasiliensis) from a Colony in Florida, USA. Diseases. 2017;5(1):7. doi 10.3390/diseases5010007.

85. Moreno A, Lelli D, De Sabato L, Zaccaria G, Boni A, Sozzi E, et al. Detection and full genome characterization of two beta CoV viruses related to Middle East respiratory syndrome from bats in Italy. Virol J. 2017;14(1):239. doi: 10.1186/s12985-017-0907-1.
86. Woo PC, Lau SK, Lam CS, Lau CC, Tsang AK, Lau JH, et al. Discovery of seven novel Mammalian and avian coronaviruses in the genus deltacoronavirus supports bat coronaviruses as the gene source of alphacoronavirus and betacoronavirus and avian coronaviruses as the gene source of gammacoronavirus and deltacoronavirus. J Virol. 2012;86(7):3995-4008. doi: 10.1128/jvi.06540-11.
87. Luo C-M, Wang N, Yang X-L, Liu H-Z, Zhang W, Li B, et al. Discovery of novel bat coronaviruses in South China that use the same receptor as Middle East respiratory syndrome coronavirus. J Virol. 2018;92(13):e00116-18. doi 10.1128/jvi.00116-18.
88. Barbour R, Davis W. Bats of America. Lexington. Kentucky University of Kentucky Press p. 1969.
89. Shankar V, Orciari LA, Mattos CD, Kuzmin IV, Pape WJ, O'Shea TJ, et al. Genetic divergence of rabies viruses from bat species of Colorado, USA. Vector Borne Zoonotic Dis. 2005;5(4):330-41. doi: 10.1089/vbz.2005.5.330.
90. Zana B, Kemenesi G, Urbán P, Földes F, Görföl T, Estók P, et al. Metagenomic analysis of bat guano samples revealed the presence of viruses potentially carried by insects, among others by Apis mellifera in Hungary. Acta Vet Hung. 2018;66(1):151-61. doi: 10.1556/004.2018.014.
91. Grozinger CM, Flenniken ML. Bee viruses: Ecology, pathogenicity, and impacts. Annu. Rev. Entomol. 2019;64:205-226. doi: 10.1146/annurev-ento-011118-111942.
92. Jánoska M, Vidovszky M, Molnár V, Liptovszky M, Harrach B, Benkő M. Novel adenoviruses and herpesviruses detected in bats. Vet J. 2011;189(1):118-21. doi: 10.1016/j.tvjl.2010.06.020.
93. Finoketti F, Dos Santos RN, Campos AAS, Zani ALDS, Barboza CM, Fernandes MES, et al. Detection of adenovirus, papillomavirus and parvovirus in Brazilian bats of the species Artibeus lituratus and Sturnira lilium. Arch Virol. 2019;164(4):1015-25. Epub 2019/02/10. doi: 10.1007/s00705-018-04129-1.
94. Sonntag M, Mühldorfer K, Speck S, Wibbelt G, Kurth A. New adenovirus in bats, Germany. Emerg Infect Dis. 2009;15(12):2052. doi: 10.3201/eid1512.090646.
95. Vidovszky MZ, Kohl C, Boldogh S, Görföl T, Wibbelt G, Kurth A, et al. Random sampling of the Central European bat fauna reveals the existence of numerous hitherto unknown adenoviruses. Acta Vet Hung. 2015;63(4):508-25. doi: 10.1556/004.2015.047.
96. Scott MK, Chommanard C, Lu X, Appelgate D, Grenz L, Schneider E, et al. Human adenovirus associated with severe respiratory infection, Oregon, USA, 2013–2014. Emerg Infect Dis. 2016;22(6):1044. doi: 10.3201/eid2206.151898.
97. Pauly M, Hoppe E, Mugisha L, Petrzelkova K, Akoua-Koffi C, Couacy-Hymann E, et al. High prevalence and diversity of species D adenoviruses (HAdV-D) in human populations of four Sub-Saharan countries. Virol J. 2014;11:25. Epub 2014/02/11. doi: 10.1186/1743-422X-11-25.
98. Benkö M, Harrach B. Molecular evolution of adenoviruses. Curr Top Microbiol Immunol. 2003;272:3-35. Epub 2003/05/16. doi: 10.1007/978-3-662-05597-7_1.
99. Davison AJ, Benkő M, Harrach B. Genetic content and evolution of adenoviruses. J Gen Mol Virol. 2003;84(11):2895-908. doi: 10.1099/vir.0.19497-0.
100. Anderson M, Higgins P, Davis L, Willman J, Jones S, Kidd I, et al. Experimental parvoviral infection in humans. Journal of Infectious Diseases. 1985;152(2):257-65. doi 10.1093/infdis/152.2.257.
101. Shackelton LA, Parrish CR, Truyen U, Holmes EC. High rate of viral evolution associated with the emergence of carnivore parvovirus. Proc Natl Acad Sci. 2005;102(2):379-84. doi: 10.1073/pnas.0406765102.

102. Shackelton LA, Hoelzer K, Parrish CR, Holmes EC. Comparative analysis reveals frequent recombination in the parvoviruses. J Gen Mol Virol. 2007;88(Pt 12):3294. doi: 10.1099/vir.0.83255-0.
103. Lukashov VV, Goudsmit J. Evolutionary relationships among parvoviruses: virus-host coevolution among autonomous primate parvoviruses and links between adeno-associated and avian parvoviruses. J Virol. 2001;75(6):2729-40. doi: 10.1128/jvi.75.6.2729-2740.2001.
104. Shi Z. Bat and virus. Protein & cell. 2010;1(2):109-14. doi: 10.1007/s13238-010-0029-7.
105. Matthijnssens J, Bilcke J, Ciarlet M, Martella V, Bányai K, Rahman M, et al. Rotavirus disease and vaccination: impact on genotype diversity. Future Microbiol. 2009;4(10):1303-16. doi: 10.2217/fmb.09.96.
106. Drexler JF, Seelen A, Corman VM, Tateno AF, Cottontail V, Zerbinati RM, et al. Bats worldwide carry hepatitis E virus-related viruses that form a putative novel genus within the family Hepeviridae. J Virol. 2012;86(17):9134-47. doi: 10.1128/JVI.00800-12.
107. Wang B, Yang X-L, Li W, Zhu Y, Ge X-Y, Zhang L-B, et al. Detection and genome characterization of four novel bat hepadnaviruses and a hepevirus in China. Virol J. 2017;14(1):1-10. doi: 10.1186/s12985-017-0706-8.
108. Kobayashi T, Murakami S, Yamamoto T, Mineshita K, Sakuyama M, Sasaki R, et al. Detection of bat hepatitis E virus RNA in microbats in Japan. Virus Genes. 2018;54(4):599-602. doi: 10.1007/s11262-018-1577-9.

Supporting information

S1 Fig. Total number of assembled reads to genomes of viruses from vertebrates and non-vertebrates in each bat species. Differentiation between non-migrating and migrating bats. The migrating bats are each plotted on the right side (*Nyctalus leisleri*, *N. nocutla*, *Pipistrellus nathusii* and *Vespertilio murinus*).



S2 Table. Overview of the genome organization of the contigs from de novo analysis.

virus	GenBank acc. number	strain name	Protein/ ORF	Position (nt)	Number of amino acids (nt)	Reference sequence
Adenovirus						
	MT815927	Bat adenovirus 2 strain PNV1/Switzerland/2019 hexon, protease, DNA-binding protein, 100K, 22k, pVIII, 12.5K, E3 region, fiber, U exon protein, hypothetical protein, 34K, ORFD, C, B, A genes, complete cds	Hexon	155 – 2’878	2’723	[1-3], NC_015932, NC_016895, NC_031948
			Protease	2’890 – 3’510	620	
			DNA-binding protein	4’939 – 3’554	1’385	
			100K	4’951 – 7’038	2’087	
			22K	6’875 – 7’405	530	
			pVIII	7’548 – 8’237	689	
			12.5K	8’224 – 8’574	350	
			E3 region/ ORF1	8’597 – 9’769	1’172	
			Fiber	9’963 – 11’636	1’673	
			U exon protein	9’964 – 9’797	167	
			Hypothetikal protein/ ORF6/7	11’890 – 11’651	239	
			34K			

		Hypothetical protein/ORF D	12’676 – 11’891	785	
			13’129 – 12’677	452	
		Hypothetical protein/ORF C	13’418 – 13’005	413	
		Hypothetical protein/ORF B	13’804 – 13’445	359	
		Hypothetical protein/ORF A	14’287 – 13’892	395	
MT815928	Bat adenovirus 2 strain PNV1/Switzerland/2019 E1A, E1B 19K, E1B 55K, IX, IVa2, DNA polymerase, pTP, 52K, pIIIa and III genes, complete cds; pVII gene, partial cds	E1A	999 – 1’208	209	[1-3]
		E1B 19K	1’377 – 1’916	539	NC_015932, NC_016895, NC_031948
		E1B 55K	1’739 – 3’073	1’334	
		IX	3’140 – 3’451	311	
		IVa2	4’665 – 3’448	1’217	
		DNA polymerase	7’754 – 4’557	3’197	
		pTP	9’685 – 7’820	1’865	
		52K	9’680 – 10’873	1’193	
		pIIIa	10’746 – 12’491	1’745	
		III	12’560 – 13’993	1’433	

		pVII	14’025 - >14’108	> 83	
MT815929	Bat adenovirus 2 strain PPV2/Switzerland/2019 E1A, E1B 19K, E1B 55K, IX, IVa2, DNA polymerase, pTP, 52K and pIIIa genes, complete cds; III gene, partial cds	E1A	826 – 1’035	209	[1-3]
		E1B 19K	1’204 – 1’749	545	NC_015932, NC_016895, NC_031948
		E1B 55k	1’566 – 2’906	1’340	
		IX	2’973 – 3’284	311	
		IVa2	4’498 – 3’281	1’217	
		DNA polymerase	7’587 – 4’390	3’197	
		pTP	9’518 – 7’653	1’188	
		52K	9’513 – 10’706	1’193	
		pIIIa	10’579 – 12’327	1’748	
		III	12’396 - >13’694	> 1’298	
MT815930	Bat adenovirus 2 strain PPV2/Switzerland/2019 hexon, protease and DNA-binding portein genes, complete cds; 100K gene partial cds	Hexon	7 – 699	692	[1-3]
		Protease	711- 1’331	620	NC_015932, NC_016895, NC_031948
		DNA-binding protein	2’760 – 1’375	1’385	
		100K	2’772 - >3’125	> 353	

MT815931	Bat adenovirus 2 strain PPV2/Switzerland/2019, III and pVII genes, complete cds; V gene, partial cds	III	103 – 1’056	953	[1-3]
		pVII	1’088 – 1’465	377	NC_015932, NC_016895, NC_031948
		V	1’533 – > 2’183	> 650	
MT815932	Bat adenovirus 2 strain PPV2/Switzerland/2019, 100K, 22K, pVIII, 12.5K, E3 region and U hexon genes, complete cds; fiber gene, partial cds	100K	25 – 1’725	1’700	[1-3]
		22K	1’562 – 2’092	530	NC_015932, NC_016895, NC_031948
		pVIII	2’235 – 2’924	689	
		12.5K	2’911 – 3’261	350	
		E3 region/ORF1	3’284 – 4’456	1’172	
		Fiber	4’650 - > 4’952	> 302	
		U exon protein	4’651 – 4’484	167	
MT815933	Bat adenovirus isolate Nynoc/Switzerland/2019 DNA polymerase (pol) gene, partial cds	DNA polymerase	31- > 1’671	> 1’640	[1-3]
					NC_015932, NC_016895, NC_031948
MT815934	Bat mastadenovirus Pnathusii/Switzerland/2019 E1B, IVa2, DNA polymerase and DNA terminal protein genes, complete cds; 52K gene, partial cds	Small T-antigen/ E1B protein	643 – 1‘095	452	[1-3]
		Large T-antigen/ E1B protein	996 – 2‘225	771	NC_015932, NC_016895, NC_031948
		IVa2	3‘311 – 2‘577	734	

		IVa2	3‘596 – 3‘366	230	
		DNA polymerase	6‘775 – 3‘650	3‘125	
		DNA terminal protein	8‘607 – 6‘937	1‘670	
		52K	8‘772- > 9‘191	419	
MT815935	Bat mastadenovirus B strain Ppipistrellus/Switzerland/2019 hypothetical protein CaV1gp02, E1B 19K, E1B 55K, IX, IV2a, DNA polymerase and pTP genes, complete cds	Hypothetical protein CaV1gp02	195 – 398	203	[1-3] NC_015932, NC_016895, NC_031948
		E1B 19K	557 – 1’084	527	
		E1B 55k	919 – 2’256	1’337	
		IX	2’327 – 2’653	326	
		IV2a	3’867 – 2’650	1’217	
		DNA polymerase	6’968 – 3’759	3’209	
		pTP	8’863 – 7’034	1’829	
MT815936	Bat mastadenovirus B strain Ppipistrellus/Switzerland/2019, pX and pVI genes, complete cds; hexon gene, partial cds	pX	350 – 559	209	[1-3] NC_015932, NC_016895, NC_031948
		pVI	613 – 1’320	707	
		hexon	1’388 - > 3’568	> 2’180	

Circovirus						
	MT815980	Bat associated circovirus strain BatACV/Mm1/Switzerland/2019, complete genome	Replicase Capsid	<1 – 885 1’523 - 900	> 885 623	[4, 5] NC_034210
	MT815981	Bat associated circovirus strain BatACV/Rh1/Switzerland/2019, complete genome	Capsid Replicase	4 – 633 1’664 - 789	629 875	[4, 5] NC_034210
	MT815982	Bat associated circovirus strain BatACV/BtVm/Switzerland/2019, complete genome	Relicase Capsid	54 – 974 2’044 – 1’265	920 779	[4, 5] NC_034210
Coronavirus						
	MT818221	Middle East respiratory syndrome-related coronavirus strain Bat CoV/V.murinus/Switzerland/2019, ORF1ab (orf1ab), ORF1a (orf1ab) and S protein genes, partial cds	ORF1a ORF1ab S protein	43 – 10’482 10’671 – 18’545 18’487 - > 20’187	10’439 7’874 >1’700	[6, 7] NC_019843 [4, 8-17]
Hepevirus						
	MT815970	Bat hepevirus BtHEV-Ps1 strain/P.nathusii/Switzerland/2019 genes for nonstructural polyprotein, capsid proteins, partial cds	Nonstructural polyprotein Capsid protein	< 183 – 4’667 <4’749 – 6’575	> 4’484 1’826	[18] NC_040835

Parvovirus						
	MT815971	Bat parvovirus strain BtNn-PV/Switzerland/2019 NS1 gene, complete cds	NS1	187 – 1’029	842	[19] NC_016744
	MT815972	Bat parvovirus strain BtPk-PV/Switzerland/2019 NS1 and VP1 genes, complete cds	NS1	9 – 2’015	2’004	[19]
			VP1	2’674 - 4’605	1’931	NC_016744
	MT815973	Bat parvovirus strain BtPn-PV/Switzerland/2019 NS1 gene, complete cds	NS1	98 – 2’086	1’988	[19] NC_016744
	MT815974	Bat parvovirus strain BtPn-PV/Switzerland/2019 VP1 gene, complete cds	VP1	203 – 1’897	1’694	[19] NC_016744
	MT815975	Bat parvovirus strain BtPp-PV/Switzerland/2019 NS1 gene, partial cds	NS1	49 - > 744	> 695	[19] NC_016744
	MT815976	Bat parvovirus strain BtPp-PV/Switzerland/2019 VP1 gene, complete cds	VP1	196 – 1’902	1’706	[19] NC_016744
	MT815977	Bat parvovirus strain BtPa-PV/Switzerland/2019 NS1 gene, complete cds	NS1	186 – 2’192	2’006	[19] NC_016744
	MT815978	Bat parvovirus strain BtPa-PV2/Switzerland/2019 NS1 gene, complete cds	NS1	60 – 2’066	2’006	[19]

						NC_016744
	MT815979	Bat parvovirus strain BtPn-PV2/Switzerland/2019 VP1 gene, complete cds	NS1	674 – 2’599	1’925	[19] NC_016744
Rotavirus A						
	MT815937	Bat rotavirus strain BatRVA/Bat-wt/CH/Pip_kuh/2019 VP1 gene, partial cds	VP1	22 - >1’008	> 986	NC_040405 – 15 [20]
	MT815938	Bat rotavirus strain BatRVA/Bat-wt/CH/Rhi_fer/2019 VP1 gene, partial cds	VP1	340 - > 3’111	> 2’771	NC_040405 – 15 [20]
	MT815939	Bat rotavirus strain BatRVA/Bat-wt/CH/Rhi_hip/2019 VP1 gene, partial cds	VP1	121 - >1’938	> 1’817	NC_040405 [20]
	MT815940	Bat rotavirus strain BatRVA/Bat-wt/CH/Pip_pip/2019 VP1 gene, partial cds	VP1	141 - > 338	> 197	NC_040405 [20]
	MT815941	Bat rotavirus strain BatRVA/Bat-wt/CH/Pip_pip2/2019 VP2 gene, complete cds	VP2	15 – 2’663	2’648	NC_040405 [20]

	MT815942	Bat rotavirus strain BatRVA/Bat-wt/CH/Rhi_fer/2019 VP2 gene, partial cds	VP2	30 - > 1’616	> 1’586	NC_040405 [20]
	MT815943	Bat rotavirus strain BatRVA/Bat-wt/CH/Rhi_hip/2019 VP2 gene, partial cds	VP2	354 - > 2’327	> 1’973	NC_040405 [20]
	MT815944	Bat rotavirus strain BatRVA/Bat-wt/CH/Rhi_fer/2019 VP3 gene, partial cds	VP3	455 - > 2’431	>1’976	NC_040405 [20]
	MT815945	Bat rotavirus strain BatRVA/Bat-wt/CH/Rhi_hip/2019 VP3 gene, partial cds	VP3	433- > 2’247	>1’814	NC_040405 [20]
	MT815946	Bat rotavirus strain BatRVA/Bat-wt/CH/Rhi_fer/2019 VP4 gene, complete cds	VP4	113 – 2’200	2’087	NC_040405 [20]
	MT815947	Bat rotavirus strain BatRVA/Bat-wt/CH/Rhi_hip/2019 VP4 gene, complete cds	VP4	97 – 2’184	2’087	NC_040405 [20]
Rotavirus H						
	MT815948	Bat rotavirus strain BatRVH/Bat-wt/CH/Myo_dau/2019 NSP1 gene, complete cds	NSP1	22 - 1’224	1’202	[21] NC_007552
	MT815949	Bat rotavirus strain BatRVH/Bat-wt/CH/Myo_dau2/2019 NSP1 gene, complete cds	NSP1	20 – 1’222	1’202	[21]

					NC_007552
MT815950	Bat rotavirus strain BatRVH/Bat-wt/CH/Myo_dau3/2019 NSP1 gene, complete cds	NSP1	34 – 1’236	1’202	[21] NC_007552
MT815951	Bat rotavirus strain BatRVH/Bat-wt/CH/Myo_dau/2019 NSP2 gene, complete cds	NSP2	24 - 914	890	[21] NC_007554
MT815952	Bat rotavirus strain BatRVH/Bat-wt/CH/Myo_dau2/2019 NSP2 gene, complete cds	NSP2	48 - 938	890	[21] NC_007554
MT815953	Bat rotavirus strain BatRVH/Bat-wt/CH/Myo_dau3/2019 NSP2 gene, complete cds	NSP2	73 – 963	890	[21] NC_007554
MT815954	Bat rotavirus strain BatRVH/Bat-wt/CH/Myo_dau/2019 NSP3 gene, complete cds	NSP3	83 - 799	716	[21] NC_007555
MT815955	Bat rotavirus strain BatRVH/Bat-wt/CH/Myo_dau2/2019 NSP3 gene, complete cds	NSP3	73 - 789	716	[21] NC_007555
MT815956	Bat rotavirus strain BatRVH/Bat-wt/CH/Myo_dau3/2019 NSP3 gene, complete cds	NSP3	33 – 833	800	[21] NC_007555
MT815957	Bat rotavirus strain BatRVH/Bat-wt/CH/Myo_dau3/2019 NSP4 gene, complete cds	NSP4	33 - 683	650	[21] NC_007557

		NSP5	58 - 600	542	[21]
MT815958	Bat rotavirus strain BatRVH/Bat-wt/CH/Myo_dau/2019 NSP5 gene, complete cds				NC_007558
		NSP5	55 - 597	542	[21]
MT815959	Bat rotavirus strain BatRVH/Bat-wt/CH/Myo_dau2/2019 NSP5 gene, complete cds				NC_007558
		NSP5	58 - 600	542	[21]
MT815960	Bat rotavirus strain BatRVH/Bat-wt/CH/Myo_dau3/2019 NSP5 gene, complete cds				NC_007558
		VP1	7 – 3’510	3’503	[21]
MT815961	Bat rotavirus strain BatRVH/Bat-wt/CH/Myo_dau/2019 VP1 gene, complete cds				NC_007548
		VP1	< 2 – 3’505	> 3’503	[21]
MT815962	Bat rotavirus strain BatRVH/Bat-wt/CH/Myo_dau2/2019 VP1 gene, complete cds				NC_007548
		VP1	1’319 - > 4’021	> 2’702	[21]
					NC_007548
MT815963	Bat rotavirus strain BatRVH/Bat-wt/CH/Myo_dau3/2019 VP1 gene, complete cds; VP6 gene, partial cds	VP6	1’281 - 91	1’190	[21] NC_007553
		VP2	43 – 2’991	2’948	[21]
MT815964	Bat rotavirus strain BatRVH/Bat-wt/CH/Myo_dau/2019 VP2 gene, complete cds				NC_007549
MT815965	Bat rotavirus strain BatRVH/Bat-wt/CH/Myo_dau2/2019 VP2 gene, partial cds	VP2	< 1 – 2’949	> 2’948	[21]

					NC_007549
MT815966	Bat rotavirus strain BatRVH/Bat-wt/CH/Myo_dau/2019 VP3 gene, complete cds	VP3	9 – 2’168	2’159	[21] NC_007551
MT815967	Bat rotavirus strain BatRVH/Bat-wt/CH/Myo_dau3/2019 VP3 gene, complete cds	VP3	9 – 2’168	3’159	[21] NC_007551
MT815968	Bat rotavirus strain BatRVH/Bat-wt/CH/Myo_dau2/2019 VP4 gene, partial cds	VP4	197 – 2’449	2’252	[21] NC_007550
MT815969	Bat rotavirus strain BatRVH/Bat-wt/CH/Myo_dau/2019 VP6 gene, complete cds	VP6	33 – 1’233	1’190	[21] NC_007553

1. Sonntag M, Mühldorfer K, Speck S, Wibbelt G, Kurth A. New adenovirus in bats, Germany. Emerg Infect Dis. 2009;15(12):2052. doi: 10.3201/eid1512.090646.

2. Li Y, Ge X, Zhang H, Zhou P, Zhu Y, Zhang Y, et al. Host range, prevalence, and genetic diversity of adenoviruses in bats. J Virol. 2010;84(8):3889-97. doi: 10.1007/s11262-018-1577-9.

3. Hackenbrack N, Rogers MB, Ashley RE, Keel MK, Kubiski SV, Bryan JA, et al. Evolution and cryo-electron microscopy capsid structure of a North American bat adenovirus and its relationship to other mastadenoviruses. J Virol. 2017;91(2):e01504-16. doi: 10.1128/JVI.01504-16.

4. Wu Z, Yang L, Ren X, He G, Zhang J, Yang J, et al. Deciphering the bat virome catalog to better understand the ecological diversity of bat viruses and the bat origin of emerging infectious diseases. ISME J. 2016;10(3):609-20. Epub 2015/08/11. doi: 10.1038/ismej.2015.138.

5. He B, Li Z, Yang F, Zheng J, Feng Y, Guo H, et al. Virome profiling of bats from Myanmar by metagenomic analysis of tissue samples reveals more novel Mammalian viruses. PLoS One. 2013;8(4):e61950. Epub 2013/04/22. doi: 10.1371/journal.pone.0061950.

6. Zaki AM, Van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med. 2012;367(19):1814-20. doi: 10.1056/nejmx130029.

7. de Groot RJ, Baker SC, Baric RS, Brown CS, Drosten C, Enjuanes L, et al. Commentary: Middle east respiratory syndrome coronavirus (mers-cov): announcement of the coronavirus study group. J Virol. 2013;87(14):7790-2. doi: 10.1128/JVI.01244-13.

8. Yinda CK, Ghogomu SM, Conceição-Neto N, Beller L, Deboutte W, Vanhulle E, et al. Cameroonian fruit bats harbor divergent viruses, including rotavirus H, bastroviruses, and picobirnaviruses using an alternative genetic code. Virus Evol. 2018;4(1):vey008. Epub 2018/03/30. doi: 10.1093/ve/vey008.

9. Lau SK, Li KS, Tsang AK, Lam CS, Ahmed S, Chen H, et al. Genetic characterization of Betacoronavirus lineage C viruses in bats reveals marked sequence divergence in the spike protein of pipistrellus bat coronavirus HKU5 in Japanese pipistrelle: implications for the origin of the novel Middle East respiratory syndrome coronavirus. J Virol. 2013;87(15):8638-50. doi: 10.1128/JVI.01055-13.

10. Luo C-M, Wang N, Yang X-L, Liu H-Z, Zhang W, Li B, et al. Discovery of novel bat coronaviruses in South China that use the same receptor as Middle East respiratory syndrome coronavirus. J Virol. 2018;92(13): e00116-18. doi: 10.1128/jvi.00116-18.

11. Lau SK, Woo PC, Li KS, Huang Y, Wang M, Lam CS, et al. Complete genome sequence of bat coronavirus HKU2 from Chinese horseshoe bats revealed a much smaller spike gene with a different evolutionary lineage from the rest of the genome. Virol. 2007;367(2):428-39. doi: 10.1016/j.virol.2007.06.009.

12. Woo PC, Wang M, Lau SK, Xu H, Poon RW, Guo R, et al. Comparative analysis of twelve genomes of three novel group 2c and group 2d coronaviruses reveals unique group and subgroup features. J Virol. 2007;81(4):1574-85. doi: 10.1128/JVI.02182-06.

13. Chu D, Peiris J, Chen H, Guan Y, Poon LL. Genomic characterizations of bat coronaviruses (1A, 1B and HKU8) and evidence for co-infections in Miniopterus bats. J Gen Virol. 2008;89(5):1282-7. doi: 10.1099/vir.0.83605-0.

14. T ang X, Zhang J, Zhang S, Wang P, Fan X, Li L, et al. Prevalence and genetic diversity of coronaviruses in bats from China. J Virol. 2006;80(15):7481-90. doi: 10.1128/JVI.00697-06.

15. Obameso JO, Li H, Jia H, Han M, Zhu S, Huang C, et al. The persistent prevalence and evolution of cross-family recombinant coronavirus GCCDC1 among a bat population: a two-year follow-up. Science China Life Sciences. 2017;60(12):1357-63. doi: 10.1007/s11427-017-9263-6.

16. Tao Y, Shi M, Chommanard C, Queen K, Zhang J, Markotter W, et al. Surveillance of bat coronaviruses in Kenya identifies relatives of human coronaviruses NL63 and 229E and their recombination history. J Virol. 2017;91(5):e01953-16. doi: 10.1128/JVI.01953-16.

17. Drexler JF, Gloza-Rausch F, Glende J, Corman VM, Muth D, Goettsche M, et al. Genomic characterization of SARS-related coronavirus in European bats and classification of coronaviruses based on partial RNA-dependent RNA polymerase gene sequences. J Virol. 2010; 84(21): 11336-49. doi: 10.1128/JVI.00650-10.

18. Reuter G, Boros Á, Tóth Z, Kapusinszky B, Delwart E, Pankovics P. Detection of a novel RNA virus with hepatitis E virus-like non-structural genome organization in amphibian, agile frog (Rana dalmatina) tadpoles. Infect Genet Evol. 2018;65:112-6. doi: 10.1016/j.meegid.2018.07.029.

19. Canuti M, Eis-Huebinger AM, Deijs M, de Vries M, Drexler JF, Oppong SK, et al. Two novel parvoviruses in frugivorous New and Old World bats. PLoS One. 2011;6(12): e29140. doi: 10.1371/journal.pone.0029140.

20. Small C, Barro M, Brown TL, Patton JT. Genome heterogeneity of SA11 rotavirus due to reassortment with “O” agent. Virol. 2007;359(2):415-24. doi: 10.1016/j.virol.2006.09.024.

21. Jiang S, Ji S, Tang Q, Cui X, Yang H, Kan B, et al. Molecular characterization of a novel adult diarrhoea rotavirus strain J19 isolated in China and its significance for the evolution and origin of group B rotaviruses. J Gen Virol. 2008;89(10):2622-9. doi: 10.1099/vir.0.2008/001933-0.

Acknowledgements

This work was funded by the Foundation for Research in Science and the Humanities at the University of Zurich (STWF-19-013).

Firstly, I would like to thank my supervisor Dr. med. vet. Jakub Kubacki for his supervision of this study, the introduction into the methodology and help with the data analysis and manuscript writing.

Then I want to thank especially Prof. Dr. Cornel Fraefel for the opportunity to work at his Institute and for his outstanding supervision and idea of this project.

Also, thanks to Prof. Dr. med. vet. Jean-Michel Hatt who was involved in the outlining process of this project and for his help with the manuscript writing.

Thank you Prof. Dr. Matthias Schweizer for co-examining my doctoral thesis.

Further, I would like to thank the Bat foundation in Zurich and all care takers of bats and colonies for their support with the sample collection, their inputs regarding the bats biology and especially Hubert Kraettli and Katja Schoenbaechler for proofreading my dissertation.

Thank you, Elisabeth Schraner, for the professional Electron microscope pictures.

A big thanks goes to the my group members, especially Tamara, Deborah, Karin, Charlotte, Julia Li, Julia Le, Jasmin, Sybille, Marco, Martina, Mahesa for their help in the lab, the very nice time outside of the lab, friendships and support.

I want to thank all members of our institute for the good time and nice atmosphere.

Lastly, I want to thank my family and specially Yves for their support and understanding.

Curriculum Vitae

Vorname Name	Isabelle Hardmeier
Geburtsdatum	15.04.1993
Geburtsort	Basel
Nationalität	Schweiz
Heimatort	Zumikon ZH
August/1997 – Juni/1999	Kindergarten, Therwil, Schweiz
August/1999 – Juni/2004	Primarschule, Therwil, Schweiz
August/2004 – Juni/2008	Sekundarschule Niveau P Schwerpunkt Mathematik, Therwil, Schweiz
August/2008 – Dezember/2011	Gymnasium Oberwil Profil B (Schwerpunkt Biologie und Chemie), Oberwil, Schweiz
20.12.2011	Matura Profil B Biologie und Chemie, Gymnasium Oberwil, Oberwil BL, Schweiz
September/2012 – August/2018	Studium der Veterinärmedizin, Universität Zürich, Zürich, Schweiz
28.12.2018	Staatsexamen vet. med. Universität Zürich, Zürich, Schweiz
Februar/2019 – Januar/2021	Anfertigung der Dissertation unter Leitung von Prof. Dr. sc. nat. ETH Cornel Fraefel am Institut für Virologie der Vetsuisse-Fakultät Universität Zürich Direktor: Prof. Dr. sc. nat. ETH Cornel Fraefel

Zürich, 06.01.2021